The Effects of Obesity and Weight Loss in Heart Failure: Imaging the Obesity Paradox using Magnetic Resonance Imaging and Spectroscopy

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The experience of undertaking my DPhil has been transformative. I have rediscovered a sense of curiosity about the physiology and pathology which we deal with on a daily basis. This has not only impacted my clinical practice, but reinvigorated my enthusiasm for continuing to ask questions and push the boundaries of our understanding in order to maximise benefits for patients.

This process could not have taken place without the input and time of a significant number of individuals, including colleagues who I am privileged to say have now become friends. However, the unwavering support of my supervisors, Prof Oliver Rider, Prof Stefan Neubauer and Prof Chris Rodgers, has been key both to the progress of the research described in this thesis, and to my personal development into a more ambitious and resilient scientist – and for that I am deeply grateful.

I would also like to take this opportunity to thank my parents, without whose commitment to my education at an early stage I would never have achieved so much, and whose attempts to understand how on earth I am still a student are much appreciated.

This thesis and the work of the past five years, are dedicated to my immediate family – to Tim, whose love and selfless support in every area of our life together has given me the opportunity to follow my dreams, and to our girls – you make it all worthwhile.

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Abbreviations and definitions

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ANP	Atrial natriuretic peptide
(γ-)ATP	(Gamma-) adenosine triphosphate
BMI	Body mass index
BNP	Beta natriuretic peptide
Cr	Creatine
CPET	Cardiopulmonary exercise test
CPT1	Carnitine palmitoyltransferase 1
CRP	C-reactive peptide
DEXA	Dual-energy X-Ray absorptiometry
ECG	Electrocardiogram
EDV	End-diastolic volume
EF	Ejection fraction
ESV	End-systolic volume
FAT/CD36	Fatty acid translocase complex
(F)FA	(Free) fatty acid
GLP-1	Glucagon-like peptide 1
(^{1}H-/^{31}P-) MRS	(Proton/phosphorus) magnetic resonance spectroscopy
Hct	Haematocrit
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HLA	Horizontal long axis
HOMA-IR	Homeostatic model assessment of insulin resistance
HR	Heart rate
k_f	Forward rate constant
(L)GI	(Low) glycaemic index
LV	Left ventricle/ventricular
(Mi-)/(MM-)CK	(Mitochondrial)/(Muscular) isoforms of creatine kinase

MR(I)	Magnetic resonance (imaging)
MTGC	Myocardial triglyceride content
$M\dot{V}O_2$	Myocardial oxygen uptake
(NT-pro)BNP	(N-terminal pro-) brain natriuretic peptide
NYHA	New York Heart Association (classification of heart failure symptoms)
PCr	Phosphocreatine
PDH	Pyruvate dehydrogenase
PET	Positron emission tomography
PPAR-α	Peroxisome proliferator-activated receptor alpha
RAAS	Renin angiotensin aldosterone system
RF	Radiofrequency
RPP	Rate-pressure product
RV	Right ventricle (ventricular)
SERCA	Sarco/endoplasmic reticulum calcium ATPase
SV	Stroke volume
TE	Echo time
TR	Repetition time
TrIST	Triple repetition tIme Saturation Transfer
$VE/\dot{V}CO_2$	Ventilatory equivalent for carbon dioxide
VLA	Vertical long axis
VLCD	Very low calorie diet
$\dot{V}O_2$	Whole body oxygen uptake

Abstract

Obesity and cardiovascular function are closely linked on a number of different levels, from the impact of the whole-body complications of obesity, to the geometric remodelling inflicted on the heart and vasculature by increased body weight, to the influence of the endocrine state of the body on intracellular signalling cascades, to the very efficiency of energy generation and transfer within the myocyte itself.

Whilst obesity is one of the main contributing risks to the development of number of different cardiovascular diseases, it has a particularly strong relationship with the development of heart failure. However, there is a counterintuitive association with survival in established disease and increasing body mass index, known as the obesity paradox. This underlines the complex relationship between the two conditions, and poses the question as to whether there is a protective physiological mechanism at play within the obese myocardium.

Magnetic resonance imaging is perfectly placed to interrogate this interplay in more detail, being the gold-standard technique for assessing a number of different parameters of cardiovascular structure and function, but also unveiling the potential for investigating cellular physiological parameters using non-invasive spectroscopic techniques.

It is known that inflexibility in substrate selection has an impact on cardiovascular function and that this plays an important role in the development of diabetic cardiomyopathy, as well as potentially the obesity phenotype. In this thesis, I use the popular Very Low Calorie Diet in a healthy obese cohort, both to assess its efficacy in treating obesity-related cardiovascular disease, and also to demonstrate the impact of

altering the availability of myocardial substrate on cardiovascular function. While after 8 weeks of dietary intervention, myocardial steatosis and diastolic dysfunction are reversed, there is an interesting early deterioration in function, associated with an increase in circulating fatty acids and deposition of ectopic lipid within the myocardium. This highlights the potential for dietary intervention as a treatment strategy in metabolic cardiac dysfunction, but also the need for caution in a population with pre-existing cardiovascular disease.

While substrate selection is an important factor in determining the efficiency of energy generation, the rate of transfer of ATP from the mitochondrion to the myofibril is also a key step in regulating myocardial energy transduction. This step is catalysed by the creatine kinase enzyme, enabling PCr to act as a buffer maintaining rates of ATP delivery under fluctuating demand. The reaction rate of creatine kinase can be interrogated with sophisticated non-invasive MR spectroscopic techniques, and has been found to be reduced in heart failure. In obesity, however, there is typically preserved systolic function despite reduced PCr/ATP concentrations, which raises the question of whether creatine kinase kinetics compensate to maintain ATP delivery. I investigated this question in obese individuals without additional cardiac disease, and found not only that the reaction rate of creatine kinase was elevated, maintaining ATP delivery at rest, but that the enzyme reaction rate response to catecholamine stress was blunted, yielding no increase in energy delivery despite increased workload. This was linked to whole body exercise capacity, and reversed with intentional weight loss. These results suggest that obesity may have direct effects on the resting energetic state in the myocardium, but that this comes at a cost of reduced reserve to cope with increased demand. This may explain why exertional symptoms are so prevalent in obesity.

To investigate the impact of obesity on myocardial energetics in individuals with established cardiac dysfunction, I examined volunteers either normal weight or obese with dilated cardiomyopathy, measuring myocardial PCr/ATP and creatine kinase kinetics at rest and under dobutamine stress, and interpreting these results in the context of cardiac function and exercise capacity. In obese heart failure, baseline PCr/ATP was lower compared to individuals with normal systolic function, but the creatine kinase rate constant was again elevated compared to those of normal weight. This meant that in obese heart failure, overall ATP delivery was maintained to a similar degree to those without systolic dysfunction. However, the diminished capacity to cope with increased haemodynamic demand was exacerbated, with catecholamine stress leading to a fall in PCr/ATP, blunted response in creatine kinase activity, and an overall fall in ATP delivery. This was in the context of poor exercise capacity, and may be linked to exertional symptoms in obese heart failure.

The impact of intentional weight loss on cardiac function and energetic status in obese heart failure was examined in the final chapter. Volunteers were asked to adhere to a calorie controlled diet for a period of 12 months before comprehensive retesting was performed. The striking finding was an improvement in contractile function, even in volunteers with established heart failure, with a marked improvement in left ventricular ejection fraction with weight loss. Equally exciting was significant cardiac remodelling, with reduction in left ventricular end-diastolic volume and mass. The changes in myocardial energetics were more subtle, with no change in PCr/ATP, creatine kinase activity at rest, or ATP delivery. However, there was a suggestion of restoration of the stress response of enzyme activity, with individuals with successful weight loss seeing an increase in stress creatine kinase kinetics which had not previously been measured. These

results raise a number of interesting points; clinically, whether intentional weight loss has the potential to become a therapeutic tool in heart failure, and on a more mechanistic level, how left ventricular contractility can improve despite no measurable change in resting energy delivery.

In summary, this thesis interrogates the mechanisms underpinning the relationship between obesity and cardiac function, demonstrating the importance of substrate selection in determining cardiovascular function, establishing a potentially compensated energetic state in the obese myocardium, and providing the first stage of evidence for intentional weight loss to be used as an additional treatment strategy in obese heart failure.

Chapter One

Introduction

1. Overview

Obesity is one of the most significant health issues in modern society, and levels continue to rise rapidly[1]. It increases the risk of developing a wide range of cardiovascular conditions, including hypertension and heart failure[2-4]. It poses a multi-hit challenge for the cardiovascular system, increasing cardiac work due to increased haemodynamic demand[5], activation of neurohormonal systems, and vascular dysfunction[6]. At the same time, the efficiency of that cardiac work is reduced, due to metabolic derangement[7], leading to a shift in myocardial substrate availability for high-energy phosphate metabolism[8]. It is unsurprising therefore, that obesity is a significant risk factor for the development of heart failure, as well as increasing the severity of the symptoms and functional limitation caused by the disease[9].

The worldwide prevalence of heart failure continues to increase steadily[10, 11], especially that of heart failure with preserved ejection fraction (HFpEF) for which obesity is one of the most significant risk factors[12]. Given that prognosis in heart failure is relatively static[13] despite gold-standard pharmacological therapy, there is increasing focus on the management of risk factors and contributory comorbidities. Hence, the management of obesity in the context of heart failure is highly relevant, and yet a paucity of evidence means that it remains a controversial field.

The obesity paradox is well-established in heart failure[14], demonstrating increased prognosis and survival in overweight and obese when compared to normal weight individuals. The potential explanations for this include differences in the sample groups, increased cardiovascular fitness, or an actual protective physiological mechanism underlying the obese state. Current international clinical guidelines for heart failure[15, 16] draw on observational retrospective data looking at change in body weight in cohorts recruited for pharmacological trials, which demonstrate that weight loss is associated with increased mortality[17-19], even in obese individuals[20]. However, crucially, these studies have no way of differentiating intentional weight loss in the modern obese, insulin resistant patient, from unintentional loss of muscle mass indicating cachexia, which is associated with significantly reduced survival[21].

Intentional weight loss in obesity in the absence of heart failure is associated with beneficial cardiac remodelling[22, 23], improved metabolic and energetic status[24, 25], and improved prognosis. There are some studies which have suggested a beneficial impact of intentional weight loss in heart failure[26, 27] – however, these have been small and limited in their scope, such that guidelines have not been impacted.

During the rest of this chapter, I will review the current evidence for the links between obesity and myocardial dysfunction, the evidence and potential mechanisms underlying the obesity paradox, and the potential for weight loss as a therapeutic tool, hence providing context for, and the potential significance of, the work described in the thesis.

2. Myocardial changes in obesity

2.1 Obesity-related structural changes in the heart

Although William Harvey observed in the 17th century that the heart in obesity was "large, thick and fibrous", global myocardial hypertrophy in obesity in the absence of hypertension was first recognised in 1933[28]. In this early series of post-mortem studies, elevated total cardiac weight/body weight ratio was demonstrated. This is important as hypertension is common in patients with obesity, and if present, even small increases in systolic blood pressure exaggerate the hypertrophic response[29]. This not only linked obesity to global cardiac hypertrophy directly, but also suggested, for the first time, that obesity itself could be a cause of heart failure (HF)[28].

It was then not until 1992 that cardiac myocyte hypertrophy in obesity was confirmed on endomyocardial biopsy in those referred for HF investigations[30]. In addition, patients with obesity were less likely to have a specific aetiology diagnosed for their HF, suggesting an obesity specific cardiomyopathy.

Excess adiposity imposes an increased metabolic demand on the body, and thus both cardiac output and total blood volume are elevated in obesity. This hyperdynamic circulation causes left and right ventricular structural changes, which were initially thought to lead to elevated ventricular mass and cavity dilatation[31, 32]. In this early model, the hypertrophic response of the ventricle was secondary to increased wall stress imposed by cavity dilatation and increased filling pressures[33, 34]. This hypothesis was mostly driven by early imaging of the heart in obesity, where cavity dilation was described in individuals

with morbid obesity[35]. However, early studies tended to have included co-morbidities such as established HF.

Recently, there has been something of a paradigm shift in the consensus regarding the pattern of hypertrophy seen in obesity. From more recent studies, investigating individuals who are obese but otherwise healthy, it is apparent that although increases in both left ventricular dimensions and wall thickness are reported, concentric rather than purely eccentric hypertrophic remodelling can occur[36-39]. In addition, a sex-specific difference in left[37, 40] and right ventricular remodelling[41] appears to occur in obesity, with males exhibiting greater concentric remodelling. It is also emerging that the distribution of body fat is important, with visceral "android" fat deposition being linked to concentric hypertrophy, and lower body "gynoid" fat associated with eccentric remodelling[42]. As concentric hypertrophy is more strongly predictive of cardiovascular mortality than eccentric[43-46], this provides a possible explanation for sex-specific mortality differences in obesity.

Unpublished data from our group has demonstrated that increasing BMI is not only associated with LV remodelling in those without coexisting cardiac disease, but also in those with a formal diagnosis of dilated cardiomyopathy (figure 1.1).

2.2 Cardiac functional changes

Although linked with heart failure on a population level[3], the majority of studies report that obesity has little or no deleterious effect on global echocardiographic measures of systolic function such as left ventricular ejection fraction[47, 48]. This suggests that, although some individuals are susceptible to developing heart failure, it is not a universal phenomenon, and on the contrary, many studies have demonstrated increased systolic

function in obesity[49]. However, with the development of more sensitive measures of early systolic impairment such as strain and strain rate, subclinical abnormalities have been found to correlate not only with BMI[29, 50-52], but also duration of obesity[53]. However, there is currently a lack of longitudinal data to establish whether these early changes progress to manifest systolic HF.

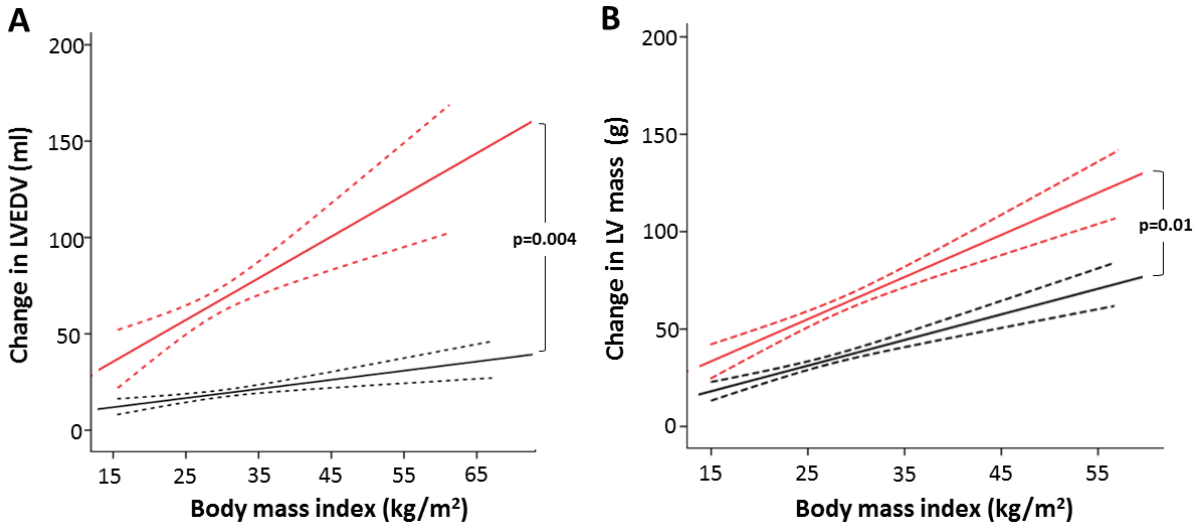


Figure 1.1: Increasing body mass has a more significant effect on left ventricular geometry in dilated cardiomyopathy (red) than normal individuals (black); unpublished registry data in n=728 (normal) and n=529 (DCM) (Rayner et al)

Early data on diastolic function and obesity proved inconsistent when assessed with mitral inflow data in isolation[54]. This is not surprising when considering that volume loading conditions exert significant influence on these measurements[55]. With the advent of more load-independent measures of diastolic function such as tissue Doppler imaging, several studies have found increasing BMI associated with deteriorating diastolic function[36, 56]. Overall, it is now universally accepted that obesity is associated with diastolic dysfunction[36, 57, 58], and is one of the main contributing factors to the development of HFpEF[4, 12, 59].

2.3 Myocardial substrate utilisation in obesity and lipotoxicity

Obesity is not only associated with abnormalities on a whole-body or organ system level, but also with metabolic and transcriptional derangement at the cellular level. The myocardium is particularly sensitive to metabolic dysregulation. In healthy adults, the myocyte is able to switch efficiently between fuel sources in order to maintain maximal work under the prevailing conditions[60] – in other words, predominantly using fatty acids under fasted, resting conditions, but utilising pyruvate from glycolysis, lactate or ketones under fed or stressed conditions. In the obese, insulin-resistant heart, the ability of the myocardium to use glucose is impaired, due in part to reduction in insulin-activated translocation of the glucose transporter GLUT4 from its intracellular stores to the cell surface membrane[61].

As well as impaired glucose uptake, obesity is associated with a dramatic shift towards fatty acid metabolism. Obesity results in activation of peroxisome proliferator-activated receptor alpha (PPARα), a major controller of myocardial substrate selection[62]. This activation of PPARα initially compensates for the elevated circulating free fatty acid levels in obesity by increasing myocardial fatty acid uptake and oxidation[63-65]. In addition, obesity results in a larger proportion of the fatty acid translocase (FAT/CD36) complex being located at the plasma membrane[66, 67] and abolishes normal insulin-mediated translocation of FAT/CD36. This suggests that a substantial portion of the FAT/CD36 pool is relocated to the sarcolemma in obesity, facilitating increased fatty acid uptake[68].

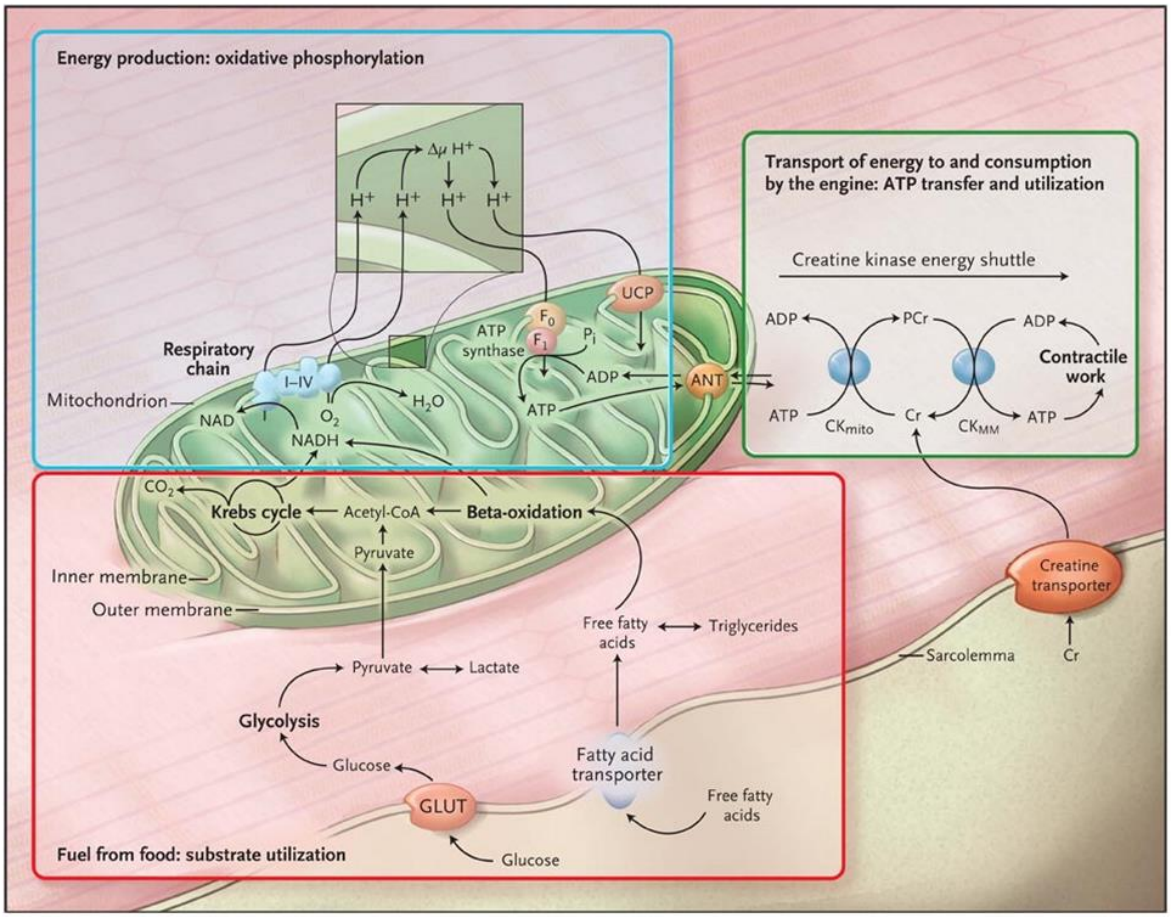


Figure 1.2: Cardiac energy metabolism. Energy metabolism in the heart has three components: substrate utilisation (red outline), energy production (blue outline) and energy transfer (green outline) (Neubauer[69])

In addition, mitochondrial fatty acid uptake (via carnitine palmitoyltransferase 1 (CPT I)) and β-oxidation rates are also elevated in experimental models of obesity and diabetes as a result of PPARα activation and pyruvate dehydrogenase inhibition[70, 71]. Whilst in the early stages, this helps to regulate intracellular level of fatty acids, eventually the increase in substrate pool overwhelms the oxidative capacity of the tissue, leading to accumulation of metabolites such as long-chain acyl CoA, diacylglycerol and ceramide. The resulting cellular dysfunction has been termed lipotoxicity[72, 73].

The defects seen in the myocardium as a result of lipid accumulation include increased reactive oxidative species[74], markers of apoptosis and cardiac dysfunction; features which

were then reversed by the reduction of cardiac lipid species[72]. The increase in intracellular lipid triggers a cascade of signalling, which leads to defects in both sensing and effectiveness of insulin[7], including inhibition of glucose oxidation via PDH (the Randle cycle[75]). However, there is also evidence that obesity is paradoxically associated with impaired fatty acid oxidation, due to a blunted response of the PPAR-α system to FAs[76] in animal models, although this is not backed up by human studies. Therefore, obesity leads to a pathological combination of intracellular insulin resistance, relative impairment of fatty acid oxidation and lipotoxicity.

One of the key cellular hubs in terms of regulating substrate utilisation, in addition to PPAR-α, is the AMP-activated protein kinase (AMPK), which among other processes regulates the balance between FA synthesis and oxidation[77]. It is activated by low ATP and high AMP, essentially sensing low energy states and activating FA oxidation to generate ATP[78]. The activity of AMPK is affected by obesity, with blunted response to leptin[79], and is a potentially both a crucial mediator of the metabolic abnormalities resulting from obesity, and also a therapeutic target.

2.4 Myocardial inefficiency and energetic depletion

The loss of metabolic flexibility and the ability to switch between substrates according to the prevailing conditions, leads to increased oxygen and substrate demand for work done; in other words, inefficient myocardial function. Although, when measured in the isoloated heart, the potential energy yielded per unit of lipid is higher than that for pyruvate[80], the actual amount of ATP delivered is lower, although this has not been demonstrated as yet *in vivo*. This is due to inability of the products of β-oxidation to utilise the whole of the electron transport chain, with 50% of electrons donated at

complex II rather than complex I, leading to a reduced electrochemical gradient for production of ATP molecules[81]. In addition, elevated intracellular FFAs leads to increased expression of uncoupling proteins[82], which allow the electrochemical gradient to leak across the mitochondrial membrane without being coupled to ATP generation. These pathways explain why perfusing an isolated heart with FFAs leads to an increased oxygen cost relative to glucose and insulin[83].

This obesity-induced myocardial inefficiency has also been demonstrated in human studies using positron emission topography (PET) techniques to look at myocardial oxygen uptake ($M\dot{V}O_2$) and fatty acid utilisation. Peterson et al[84] found that obesity was associated both with increased fatty acid uptake and increased $M\dot{V}O_2$ relative to cardiac work done – in other word, decreased myocardial efficiency. Both myocardial efficiency and substrate utilisation were also reversible with weight loss, resulting in measurable functional improvement[85].

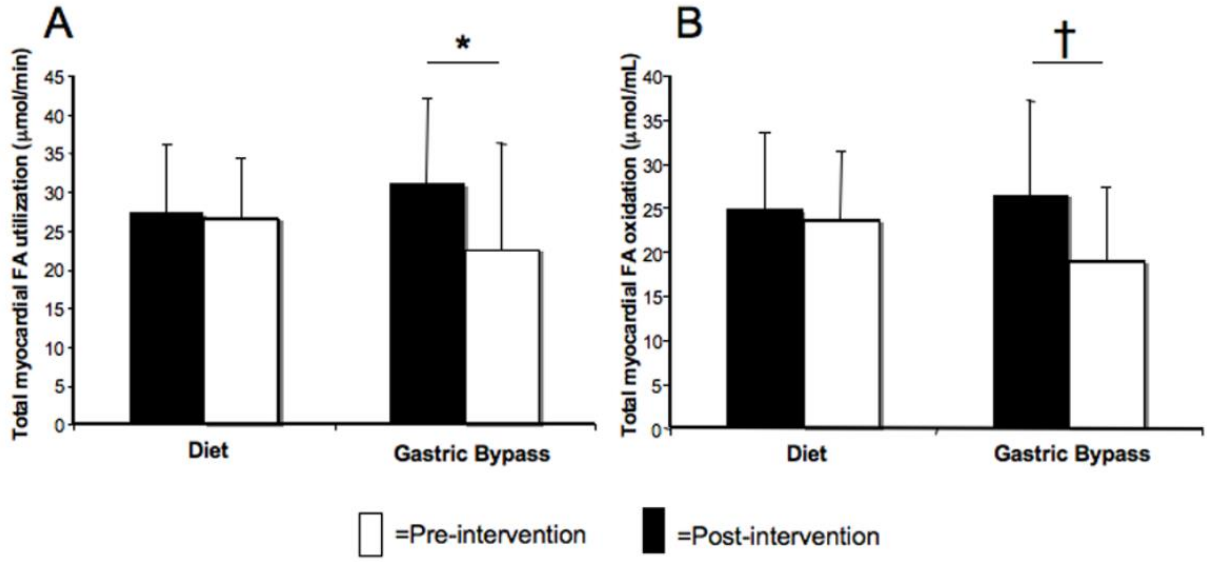


Figure 1.3: Changes with myocardial substrate utilisation with weight loss. Weight loss results in reduction in myocardial FA utilisation and oxidation (Lin et al)

These abnormalities at various stages of substrate uptake, oxidation and mitochondrial efficiency are reflected in the final unifying stage of energy production in the myocyte – the pool of ATP itself as well as its substrates, and the rate of its transfer to the contractile machinery.

The 'phosphocreatine shuttle' theory states that ATP is transferred from the mitochondrion to the myofibril, by utilising the rapid diffusion of the phosphocreatine (PCr) molecule[86]. The transfer of a phosphate group to creatine in the mitochondrion where ATP concentrations are high, is catalysed by the mitochondrial isoform of creatine kinase (Mi-CK). Once PCr reaches the myofibril, facilitating the diffusion of the high-energy phosphate group, ATP is reformed by the muscular isoform of CK (MM-CK), hence the rate of ATP delivery is determined not only by the rate of cellular respiration, but also by the activity of the CK enzyme.

It has been possible for some time now to measure a ratio of relative pool sizes of PCr and ATP using ^{31}phosphorus magnetic resonance spectroscopy, and this has been used as a marker of the energetic state of the myocardium. During periods of net energy consumption, PCr acts as a temporal buffer, maintaining ATP levels whilst its own concentration falls. In addition, ADP levels necessarily rise, which has its own intracellular adverse consequences. A low myocardial ratio of PCr/ATP has been demonstrated in numerous diseases, including dilated cardiomyopathy[87-89], hypertrophic cardiomyopathy[90], type II diabetes[91], atrial fibrillation[92] and obesity[8].

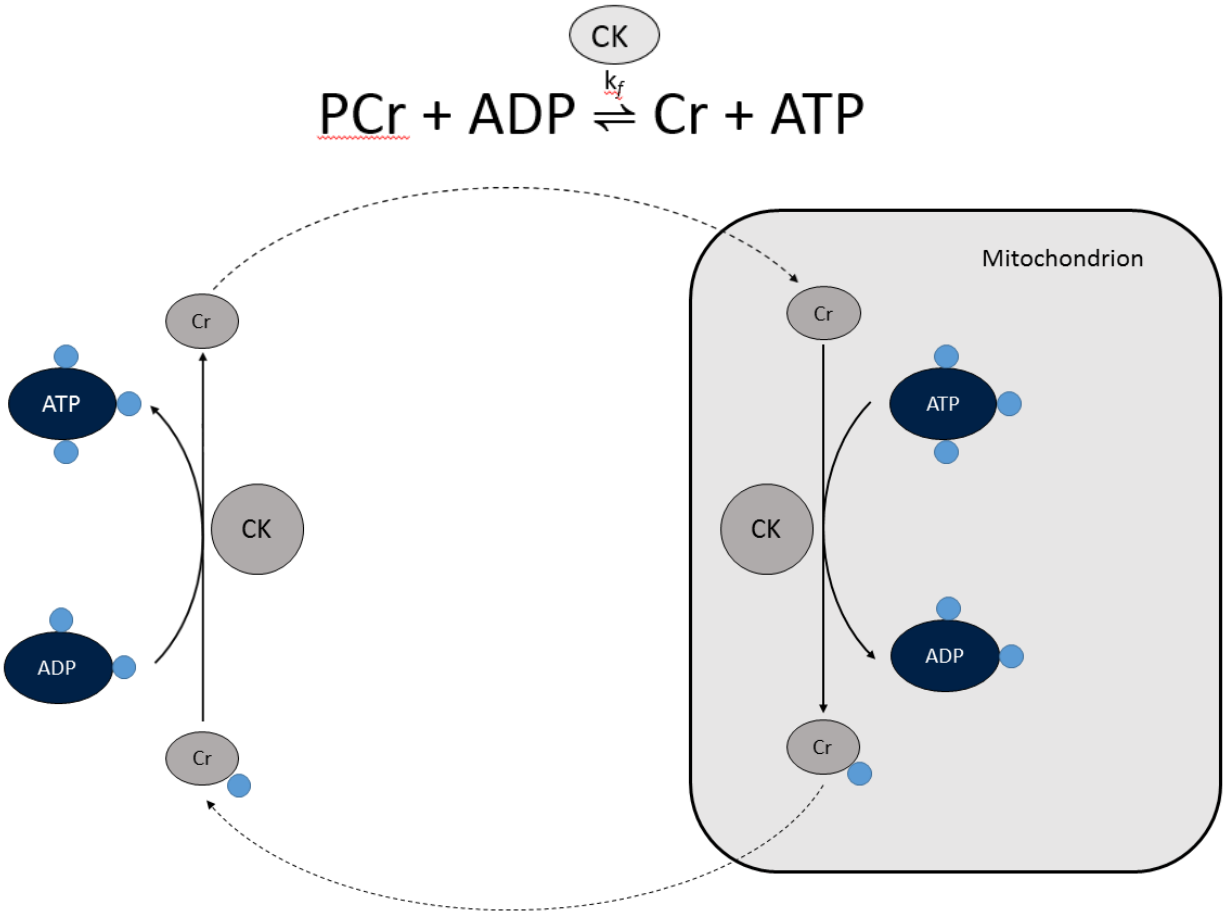


Figure 1.4: Creatine kinase (CK) catalyses the transfer of a phosphate group between creatine (Cr) and ADP in order to allow phosphocreatine to act as a temporal and spatial buffer for ATP, with a forward rate constant of k_f

However, as mentioned this only represents one element of the phosphocreatine shuttle. Utilising specific spectroscopic techniques, rates of creatine kinase activity can be non-invasively interrogated, and this has been demonstrated to be reduced in dilated cardiomyopathy[93], and myocardial infarction[94]. The measurement can differentiate more sensitively between individuals with structural cardiac disease and those with symptoms than PCr/ATP[95], and has additionally been associated with worse clinical outcomes in heart failure[96]. However, rates of creatine kinase kinetics have not as yet been assessed in obesity.

3. Adipose tissue as an endocrine organ

The past 30 years have seen a distinct change in the view of the function of adipose tissue, with the emergence of complex networks of secreted proteins with endocrine and paracrine function, demonstrating that it is in fact a highly active metabolic organ, and one with important implications for cardiovascular function. I will focus on a small selection of relevant adipocyte-derived proteins, but it is a rapidly expanding field.

3.1 Leptin

Leptin was one of the first 'adipokines' to be described, with its gene[97] and receptor[98] being identified in the mid 1990's. It is a polypeptide with structural similarities to cytokines, and is secreted by adipocytes in proportion to the amount of adipose tissue. Receptors are widely expressed, including in the myocardium. Leptin's most well-known actions are as a signal of nutrient sufficiency, leading to satiety and appetite suppression (hence the leptin-deficient mouse is often used as a model for obesity); however, it also plays an important role in the regulation of adipose tissue deposition, increasing fatty acid oxidation in non-adipose tissue including the myocardium to prevent steatosis in nutrient excess[99]. Mice which overexpress acyl CoA synthetase, and hence have increased FA uptake, develop lipotoxic heart disease with significant systolic dysfunction[100]. The steatosis and dysfunction were reversed by increasing expression of leptin[101], consistent with its role in increasing FA oxidation and preventing lipotoxicity.

However, diet-induced obesity is almost ubiquitously associated with elevated rather than reduced levels of circulating leptin, suggesting a degree of leptin resistance rather than deficiency. This has been demonstrated via a variety of purported pathways[102]

including reduced receptor expression, competing intracellular signalling pathways, binding by extracellular factors including CRP, suggesting an interaction with inflammatory pathways[103].

Clearly, obesity-induced leptin resistance has the potential to lead to a reduction in appropriate peripheral fat deposition, as well as reduced fatty acid oxidation, and hence exacerbate lipotoxicity[104]. In addition, some experimental studies have linked leptin to ventricular hypertrophy[105] and hence it has been postulated to contribute to the obesity cardiomyopathy.

In heart failure, circulating leptin levels are increased, along with increased myocardial expression of both leptin and leptin receptors; findings which are reversed by offloading mechanical stress on the ventricle with a ventricular assist device[106]. Increased leptin levels correlate with severity of heart failure symptoms as measured by the New York Heart Association classification[107], as does BMI.

3.2 Adiponectin

This more recently discovered adipokine has generated significant interest as a potential therapeutic target in heart failure. Adiponectin is secreted predominantly by adipocytes, but also in cardiac and skeletal muscle[108]. It enhances insulin sensitivity, is anti-inflammatory and promotes weight loss; however, as obesity and insulin resistance arise[109], levels of circulating adiponectin fall, whereas if insulin sensitivity increases, adiponectin levels rise[110].

Adiponectin has been found to have direct effects on cardiac myocyte metabolism, inducing glucose uptake, and fatty acid uptake and oxidation[111]. Its intracellular action

is mediated via the crucial intracellular AMPK cascade, as is the effect of leptin[79]. This increase in fatty acid metabolism has been demonstrated to reduce triglyceride accumulation in skeletal muscle[112], which if replicated in the myocardium might result in reduced lipotoxicity. In adiponectin-deficient mice, left ventricular hypertrophy was induced, and replacement of adiponectin caused reverse remodelling[113]; this association between low adiponectin levels and left ventricular hypertrophy has also been demonstrated *in vivo*[114].

The potential for utilising adiponectin as a potential treatment in metabolic heart failure is complicated by the fact that myocardial expression of adiponectin and its receptor are suppressed in heart failure[108]. This relative resistance leads to increased circulating adiponectin levels in heart failure, and this is associated with increased mortality[115]. The picture is further complicated by the recent and fascinating discovery that there is paracrine cross-talk between epicardial adipose tissue, cardiac vasculature and the myocardium[116], suggesting that further work is required before this pathway can be utilised.

3.3 Natriuretic peptides

Although obviously not an adipokine, natriuretic peptides are a fascinating example of the complex interaction between adipose tissue and myocardial function. Cardiac natriuretic peptides are released by myocytes in response to stretch, with atrial natriuretic peptide (ANP) preferentially released in the atria, and B-type natriuretic peptide (BNP) along with its biologically inactive partner NT-proBNP, more in the ventricles especially in disease states. They have widespread physiological impact, with effects on the sympathetic nervous system, renin-angiotensin system, vascular tissue, and renal

vasculature leading to increased glomerular filtration rate and therefore salt and water loss[117, 118]. There is close interaction with inflammatory cytokines – levels are also increased in renal failure, liver cirrhosis, pulmonary hypertension, and hypertension. BNP levels are significantly increased in heart failure[119] although the physiological response to the peptides seem to lose efficacy suggested a blunting of the normal pathways[117]. BNP levels in heart failure are proportional to cardiovascular events and mortality; this has been demonstrated in numerous studies[120, 121].

However, interestingly increasing BMI is inversely proportional to BNP levels – a review by Clerico et al[118] cited 18 separate studies that demonstrated different aspects of this relationship. Several potential mechanisms for this have been suggested. These include increased glomerular filtration rate in obesity leading to increased renal excretion of the peptide, increased expression of C-type natriuretic peptide receptors in adipose tissue leading to increased peripheral turnover of BNP, interactions between BNP and adipokine expression, and indirect effects via hypertension or antihypertensive drug effects on BNP [118]. Another potential mechanism turns the relationship on its head, suggesting in fact that BNP itself is lipolytic, and hence it is low levels of BNP which promote obesity, rather than the other way round[117]. Weight loss, either surgical[122] or attained through lifestyle intervention[123], is associated with increase in BNP levels.

Nonetheless, this inverse relationship complicates the investigation of heart failure in the context of obesity, especially in HFpEF, both through reluctance to accept the diagnosis in the absence of elevated BNP, and due to exclusion of obese individuals from clinical trials due to BNP being an entry criterion[124].

3.4 The sympathetic nervous system

The role of the sympathetic nervous system in mediating the pathophysiological complications of obesity has been long-established, and yet remains somewhat contradictory. Obesity is in general associated with an increase in sympathetic tone, as demonstrated by increased urinary norepinephrine[125], and efferent sympathetic signals to muscle vasculature[126], and therefore closely implicated in the development of both hypertension and heart failure. This supports the theory that this is an adaptive response in order to increase energy expenditure and maintain steady body weight[127]. Potential mediating pathways for this overactivity include obstructive sleep apnoea, impaired baroreflex activation, and insulin resistance itself[128].

However, the relationship between the autonomic nervous system and leptin secretion is more complex. One of the classical hypothalamus-mediated actions of leptin, other than inducing satiety, is to increase energy expenditure through activation of the sympathetic nervous system, mediating both lipolysis and thermogenesis[129]. This complex interplay between leptin and the sympathetic innervation of adipose tissue was elegantly interrogated in a recent study[130], in which increased sympathetic neuronal discharge was associated with facilitation of leptin-induced lipolysis.

However, in the majority of obese subjects, there is leptin resistance which should lead to reduced sympathetic tone. One theory is that leptin resistance is variable and tissue specific, with blunting of lipolysis and yet maintenance of sympathetic stimulation[131]. What is certainly differentiated is the site of increased sympathetic activity, which is particularly marked in muscle, kidney (exacerbating activation of the RAAS system), and the heart, and yet limited in terms of the lipolytic response. One particularly interesting

confounding factor in the mediation of this relationship is fat distribution, with abdominal visceral fat being more associated with sympathetic overactivity, and no such relationship being observed for subcutaneous adipose tissue[132]. This clearly demonstrates that there is more to investigate in terms of characterising obesity in order to assess its neurohormonal, and therefore cardiovascular, impact.

4. The obesity paradox in heart failure

It has been demonstrated repeatedly over the past 20 years that increased body weight is associated with improved morbidity and mortality in various chronic disease processes, including end-stage renal failure[133], rheumatoid arthritis[134] and chronic obstructive pulmonary disease[135]. Within cardiovascular disease, the obesity paradox has been reported in both coronary artery disease[136, 137] and HF[14]. This seem especially counterintuitive in setting of HF as obesity itself is a powerful, independent risk factor for developing HF and therefore it would seem logical to assume that it would be a negative prognostic factor.

Despite this, in addition to the several individual studies detailed below, robust meta-analysis of several studies (including >28,000 patients) confirms the existence of this apparent protective effect of obesity in established HF[14]. This improved prognosis occurs in both men and women[138-140], in acute HF[141-143], and occurs irrespective of country of report, ethnicity[142-144], age[145] and also importantly, in HF with both reduced and preserved ejection fraction[46, 146, 147]. Although a relatively consistent report of improved prognosis exists up to a point, some studies suggest that when morbid obesity exceeds a BMI of 40kg/m^2 this is again associated with worsened prognosis[146, 148]. In addition,

several studies have found that the protective effect is attenuated, but not lost[149, 150] in HF of ischaemic aetiology. A similar picture was observed in diabetes and HF, where obesity did not improve prognosis, but again counterintuitively was not correlated with deterioration[151, 152].

Although it is clear that the obesity paradox in HF exists in the reported studies, whether there is truly a protective influence that is exerted by obesity or whether it is simply a statistical phenomenon is the subject of active debate.

4.1 Arguments against the obesity paradox

The vast majority of studies investigating this phenomenon have been retrospective, with many exhibiting less than perfect age and comorbidity matching. Many studies report the obese groups to be younger, predominantly female, with better left ventricular systolic function, less valve disease and ischaemic heart disease, and better renal function[153]. This prompts the question as to whether the paradox reflects the survival of inherently different cohorts, which would have had different prognosis irrespective of BMI.

The majority of the studies reporting the paradox have used BMI as the metric of obesity. However, whether this is an adequate measure of obesity is much less clear, given that it does not reflect either body fat composition or distribution. When other measures of obesity have been used to assess prognosis in HF, more conflicting results are seen. When using fat mass assessed by DEXA in the HF population, lower fat mass was associated with a positive risk factor profile, although outcome data using DEXA is lacking[154]. On the other hand, alternative studies with outcome data have shown both increased skinfold

thickness and increased body fat percentage to be independently associated with a reduction in adverse events, again in line with the obesity paradox[155].

Interestingly, neither fat distribution, lean mass nor waist circumference, indirectly reflecting visceral adiposity, have consistently been able to predict mortality in the obese HF population [156, 157].

Given the relationship between obesity and breathlessness, it has also been argued that obesity may unmask HF symptoms earlier. Under those circumstances, obesity would merely highlight a group of patients at an early stage in the disease trajectory, thus introducing a form of "lag time bias", artificially lengthening the time from diagnosis to death [14, 158]. It is also possible that the common co-existence of hypertension allows more aggressive pharmacological HF treatment to be used, and better tolerated in patients with obesity[17].

In addition, it has been suggested that selection bias may exist in this population, in that more susceptible individuals succumb to comorbidities before going on to develop HF, and so only the obese individuals who are 'healthiest' are seen with HF[14]. Furthermore, as being underweight is linked to a worsened prognosis in HF[21], it is also possible that the observed survival benefit of obesity actually reflects a reverse statistical phenomenon whereby the mortality rates in individuals with obesity are flattered by a worsened mortality rate in the normal weight cohorts, which would include subjects who are cachectic due to advanced HF.

4.2 Arguments in favour of the obesity paradox

On the counter to these arguments, it should also be considered that there may indeed be intrinsic differences in the physiology of obesity which are protective once HF has developed. It is clear that being underweight with HF is a negative prognostic marker. Once advanced HF becomes catabolic leading to loss of muscle mass and 'cardiac cachexia', it carries a very poor prognosis[21]. It may be that having increased metabolic reserves in the form of adipose tissue delays this catabolic stage, preserving the fuel supply for the myocardium. In support of this idea, myocardial substrate handling is profoundly different in obesity as opposed to normal weight, but further investigation of the impact of adiposity on substrate utilisation in HF is needed to establish whether this could indeed be a protective mechanism.

It has been demonstrated that adipokines are implicated in a wide range of physiological processes, and it may be this balance which is protective in HF and obesity. The changes in levels of circulating leptin and adiponectin seen in obesity are associated with improved prognosis[102, 115, 159], and while it may be that these markers are merely reflecting the degree of adiposity, it may be that they themselves play a role in the paradox. Increasing leptin levels are associated with improved prognosis in heart failure[107]. It has been suggested that it is leptin acting as a surrogate measurement of adiposity which determines prognosis – in other words, that loss of adipose tissue in the catabolic stage of advanced heart failure leads to a fall in leptin levels. Therefore, leptin has been postulated as playing a part in the obesity paradox in heart failure.

High circulating levels of adiponectin are associated with mortality in heart failure. However, adiponectin is reduced in obesity through feedback inhibition of its

expression[159]; this is particularly the case when obesity is coupled with insulin resistance[112]. Weight loss increases adiponectin levels[160]. This difference in expression between heart failure and obesity states may indicate a role for adiponectin in mediating the obesity paradox. Similarly, NT-proBNP and BNP levels are known to correlate strongly with mortality in HF[120, 121], and levels of both are reduced in obesity[118], with potential implications for a protective mechanism.

An alternative, attractive hypothesis, is that obesity, up to a point, may lead to increased levels of cardiorespiratory fitness, and it is this which improves cardiovascular outcomes. It has been hypothesised that obesity impacts both central cardiac and respiratory function as well as peripheral skeletal muscle, increasing strength via a subclinical training effect, and that this could account for the improved survival[161-167].

Cardiorespiratory fitness is known to correlate closely with prognosis in healthy people irrespective of BMI. In heart failure, performance during cardiopulmonary exercise testing (CPET) is used to predict outcome. It was originally demonstrated in coronary disease that cardiorespiratory fitness attenuated the obesity paradox – low cardiorespiratory fitness is a significant factor in determining poor prognosis, and amongst groups with low fitness, high BMI is a protective factor[168]. However, results were less clear for groups with high fitness, with some results suggesting no influence for BMI and others suggesting a persistent paradox.

These results were reiterated in chronic heart failure by Lavie et al in 2013[161] - patients with systolic heart failure and varying BMIs were divided into low and high fitness groups according to $\dot{V}O_2$ max. The obesity paradox was more pronounced amongst groups with low cardiorespiratory fitness than in fitter patients, with there being no discernible

protective effect of high BMI in the high fitness group. Again however, high BMI was not associated with a poorer prognosis.

These findings were supported by a study looking at six-minute walk test to determine physical fitness, which found that the obesity paradox was more pronounced in the low fitness group and attenuated, but not reversed, in higher fitness levels[162].

A further study examined the influence of ventilatory efficiency, or $VE/\dot{V}CO_2$ slope, which may be more sensitive in terms of predicting clinical outcome than $\dot{V}O_2$ max[163]. Obese patients had significantly lower $VE/\dot{V}CO_2$ slopes, as well as better prognosis. The group suggested that this may due to early presentation in obesity with less severe disease, or that it may be related to abnormalities in lung function in obesity. They also found that cardiorespiratory fitness outperformed BMI in predicting risk, and that actually BMI was not related to outcome in their multivariate analysis.

Various mechanisms by which cardiorespiratory fitness can impact prognosis have been proposed. These include reduction in central obesity and metabolically active visceral fat, with concurrent changes in adipokine profile. There are anti-inflammatory effects with some studies associating physical activity levels with improved inflammatory mediator profile. There may also be additional impact on blood pressure, insulin sensitivity and lipid profile[169].

The impact of muscle mass and strength has also been studied in heart failure with cachectic patients displaying lower muscle mass and strength, supporting the notion of a catabolic state in severe disease[164]. A study in 2013 looking at patients with systolic heart failure found that increasing BMI was associated both with increasing fat mass and with

increasing muscle strength[165]. However, physical fitness as determined by $\dot{V}O_2$ max and $VE/\dot{V}CO_2$ slope was no different between BMI groups. This group suggested that the protective effects of increasing BMI may be related to either muscle strength or cardiovascular fitness.

5. The role of weight loss in heart failure

Despite the huge volume of publications on the obesity paradox, this remains a substantial unanswered question. There has been surprisingly little investigation as to the impact of weight loss in heart failure and whether or not this should be recommended in the face of the apparent survival benefit conferred by increasing body weight. The international guidelines give no direct recommendation, with the American Heart Association guidelines[16] commenting on obesity and insulin resistance as causes for heart failure, and advising avoiding obesity in general. They also refer to the lack of large-scale trials regarding weight loss in heart failure, and make no recommendation accordingly. The European Society of Cardiology[15] goes further, stating that weight loss in moderate obesity (BMI<35 kg/m^2) cannot be recommended. The basis of these recommendations is several retrospective analyses of pharmacological trials[17-20] which demonstrate weight loss associated with poor prognosis. However, and importantly, these studies will have included unintentional weight loss due to catabolic processes associated with advanced heart failure, and does not address the separate issue as to the effects of intentional weight loss in the overweight HF patient.

There have been studies looking at the impact of weight loss on cardiac morphology, both in normal ejection fraction as well as small, mainly retrospective analyses in heart

failure – however, there is little in the way of large-scale longer-term data regarding the effect on prognosis.

5.1 Weight loss in the absence of heart failure

There are several studies looking at the impact of dietary modification on cardiac morphology in the obese without heart failure. Haufe et al instigated a low-calorie diet in 170 patients with BMI between 25 and 45 kg/m^2, with weight loss of up to 11kg seen[23]. They found that LV mass fell, as did systolic blood pressure, but that there was no impact on systolic or diastolic function. In an additional small study, the same group demonstrated that a relatively small degree of weight loss led to a significant reduction in myocardial triglycerides when assessed by proton spectroscopy[24].

Another study looked at the impact of dietary weight loss not only on LV morphology and function, but on myocardial energetics[25]. Weight loss led to improvements in LV mass, end diastolic volume and stroke volume. Additionally, it was demonstrated that the PCr/ATP ratio was low in obesity suggesting impaired energetics, and that this improved after a period of weight loss. This correlated with an improvement in diastolic function.

More has been published regarding the impact of bariatric surgery on the cardiovascular system, with one recent meta-analysis reviewing 27 studies which had used echocardiographic assessment of patients pre- and post-surgery[170].

An early study looking at the impact of bariatric surgery found that weight loss led to improvement in systolic function in patients in whom there was already a degree of systolic dysfunction (as measured by fractional shortening on echocardiography); LV

volumes also reduced[22]. A further study based in Italy utilised novel tissue Doppler echo techniques to identify early subclinical systolic dysfunction, and found that this improved significantly following weight loss surgery[171].

There have been some very interesting investigations looking at the changes in the metabolic profile in the immediate aftermath of bariatric surgery. There is a dramatic improvement in insulin sensitivity particularly in the liver, within a very short timeframe, and certainly preceding any reduction in adipose tissue[172]. This may be related to a combination of energy restriction leading to increased hepatic insulin sensitivity, and increased insulin secretion due to a rise in release of gut hormones such as GLP-1 and peptide YY[173]. Inflammatory markers also fall rapidly[58].

Leptin levels fall rapidly, halving within the first week, and adiponectin increases steadily in keeping with reduction in adipose tissue[174]. This state promotes insulin sensitivity and ongoing long-term weight loss, as well as being anti-inflammatory. It would be attractive to postulate that the biochemical and adipokines changes seen in the short and medium term after bariatric surgery would have beneficial effects on the myocardium, and be reflected in improved function.

Numerous studies have demonstrated improvements in diastolic dysfunction following bariatric surgery using a variety of assessment tools[25, 170]. This correlates with the observation that obesity is commonly associated with diastolic dysfunction, and with the hypothesis that this is caused by the obesity itself.

5.2 Weight loss in heart failure

There is a significant paucity of well-conducted meaningful trials of intentional weight loss in heart failure; however, several small studies[26, 27] have demonstrated safety, and improved quality of life, albeit in the absence of any measured parameter of cardiac function.

There have been no large-scale trials of the impact of bariatric surgery in patients with clinical heart failure. However, there have been retrospective registry reviews[175] with promising results there have been case reports with encouraging results, with weight loss associated with borderline improved LV function. Additionally, case reports arise describing the combined implantation of gastric banding and left ventricular assist device. This was reported to lead to significant weight loss as well as improved LV dimensions, although it would have been interesting to know whether there was a clinical improvement in heart failure[176].

A small case-controlled cohort study has looked at the impact of bariatric surgery on heart failure with promising results[177]. The details for 12 heart failure patients who had undergone bariatric surgery were examined retrospectively and then compared to matched controls who underwent dietary intervention only. It was found that not only was surgery feasible and safe, but that significant weight loss was associated with both improved ejection fraction (from a mean of 22%±7% to 35±15% ($p=0.005$)) and improved NYHA class. These findings were not replicated in the control group.

6. Remaining unanswered questions

There are numerous unanswered questions in the complex, multisystem interaction between adipose tissue and cardiovascular function. Of particular interest is the apparent obesity paradox, and how increasing adiposity can be associated with both increased likelihood of developing heart failure, and yet be protective in the long term. With the aim of beginning to address this, over the course of this thesis, I will explore in more detail the following questions:

6.1 The effect of manipulating myocardial substrate utilisation with diet in obese individuals

It is clear from experimental data that altering myocardial substrate utilisation can have profound effects on cardiovascular function. One potential strategy for altering myocardial substrate utilisation is the newly resurgent Very Low Calorie Diet, and yet its impact on myocardial metabolism is unestablished. Whether this has any meaningful effect on cardiovascular function and whether this strategy has a role as a therapeutic intervention targeting the cardiovascular complications of obesity, is addressed in Chapter 3.

6.2 The impact of obesity on myocardial creatine kinase kinetics

Although it is established that obesity is associated with impaired myocardial energetics in terms of PCr/ATP, the kinetics of the phosphotransfer mechanism have yet to be interrogated in obesity. Understanding the cellular mechanisms involved in energy generation and transfer may yield further understanding of the relationship between

obesity and cardiovascular function, and indeed the obesity paradox itself. This is addressed in Chapter 4.

6.3 The effect of obesity in heart failure

In order to investigate the apparent protective effect of obesity in heart failure in more detail, the impact of obesity on heart failure with reduced ejection fraction, in terms of cardiac geometry, function and metabolism including creatine kinase kinetics, are investigated in Chapter 5.

6.4 The impact of weight loss in obese heart failure

Finally, the main unanswered question in the field will be addressed in Chapter 6 – namely, the effect of intentional weight reduction in obese heart failure with reduced ejection fraction.

Chapter Two

Methods

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1. Ethical considerations

All studies in this thesis received favourable ethical opinion from the South Central Oxford B Research Ethics Committee (REC reference 15/SC/0004) on behalf of the National Research Ethics Framework in England, and complied with the Declaration of Helsinki. Approval was also provided by the Oxford University Hospitals NHS Foundation Trust. All participants gave written informed consent for the studies.

2. Study participants

Heart failure patients were identified through clinics and clinical imaging lists at the Oxford University Hospitals NHS Foundation Trust. Patients who met the inclusion criteria were invited to receive an information leaflet. Following perusal of the leaflet and if in agreement, they were enrolled into the study.

Healthy control volunteers were recruited through poster advertising within the Oxford University Hospitals NHS Foundation Trust, or University of Oxford. They responded to advertising indicating their interest in taking part, and following initial screening for eligibility, were provided with an information leaflet. If they remained interested in the study, they were invited to join.

3. Inclusion criteria

3.1 All participants

- Male or female, aged 18-85 years
- Able and willing to give informed consent and comply with study requirements

3.2 Normal weight healthy controls

- No history of cardiac disease or diabetes
- BMI 18.5-24.9 kg/m^2

3.3 Normal weight heart failure patients

- Heart failure determined by LV ejection fraction 25-45%
- BMI 18.5-24.9 kg/m^2

3.4 Obese normal systolic function volunteers

- No history of cardiac disease
- BMI>27.5 kg/m^2

3.5 Obese heart failure patients

- Heart failure determined by LV ejection fraction 25-45%
- BMI>27.5 kg/m^2

4. Exclusion criteria

4.1 All participants

- Any contraindication to MR studies, such as implantable devices or other metal implants, internal defibrillator, cranial aneurysm clips, metallic ocular foreign bodies)

- Significant claustrophobia
- History or imaging evidence of previous myocardial infarction
- Severe valvular heart disease (> moderate stenosis or regurgitation of any valve)
- NYHA class IV heart failure
- Uncontrolled atrial fibrillation (resting rate >100bpm)
- Pregnancy or breastfeeding
- Standard contraindications to dobutamine administration, such as left ventricular outflow tract obstruction, uncontrolled hypertension, hypersensitivity to any component of drug, or previous reaction to dobutamine
- Inability, or loss of ability, to give informed consent

5. Baseline clinical assessments

At the first study visit, following the procedure for informed consent, all participants underwent a further screen for eligibility according to the criteria outlined above. In addition, they underwent:

- Assessment of drug history including allergies
- Non-invasive blood pressure assessment measured, according to standardised methods (average of 3 supine measurements with a manual sphygmomanometer, Carescape V100, GE Healthcare).
- Body composition was analysed by bio-impedance assessment (InBody 770, InBody Co Ltd, South Korea)
- A standard 12-lead electrocardiogram was recorded
- Fasting venous blood was drawn to measure circulating biomarkers. Insulin resistance was measured with the HOMA-IR model ((fasting glucose x fasting insulin)/22.5).

Blood volume was calculated from haematocrit and body weight, according to the formula (1-Hct)*(x + (y*weight in kg)), where x is 1530 for men and 864 for women, and y is 41 for men and 47.9 for women)[178].

6. Magnetic resonance imaging

Participants underwent MR imaging according to the following sequences. The exact combination varied according to the specific study involved. All imaging and spectroscopy was performed on a 3 Tesla system (Siemens Trio, Erlangen, Germany) as described below.

All images were acquired with the participant supine, and in end-expiration to minimise the effects of respiratory motion, except where indicated otherwise. All imaging was cardiac-gated, and care was taken with positioning of ECG electrodes to minimise mis-triggering. All imaging was analysed off-line, using cmr42 processing software (version 5.1.1, Circle Cardiovascular Imaging Inc, Calgary, Canada) except where specified otherwise.

6.1 Ectopic fat distribution

6.1.1 Abdominal visceral fat mass

Following sagittal localisers, abdominal visceral fat area was measured with a single breath-hold, 10mm transverse slice at the level of the 5th lumbar vertebral body, using a water-suppressed T1 weighted turbo spin echo (TSE) sequence (turbo factor 5, echo time TE 12ms, TR 200ms)[48]. This was analysed manually to provide an abdominal visceral fat area (figure 2.1). Single-slice MR assessment of abdominal fat distribution has

been well-validated to predict the total fat mass within a compartment[179]; however, this was in a cohort of men, and its applicability in women is less established.

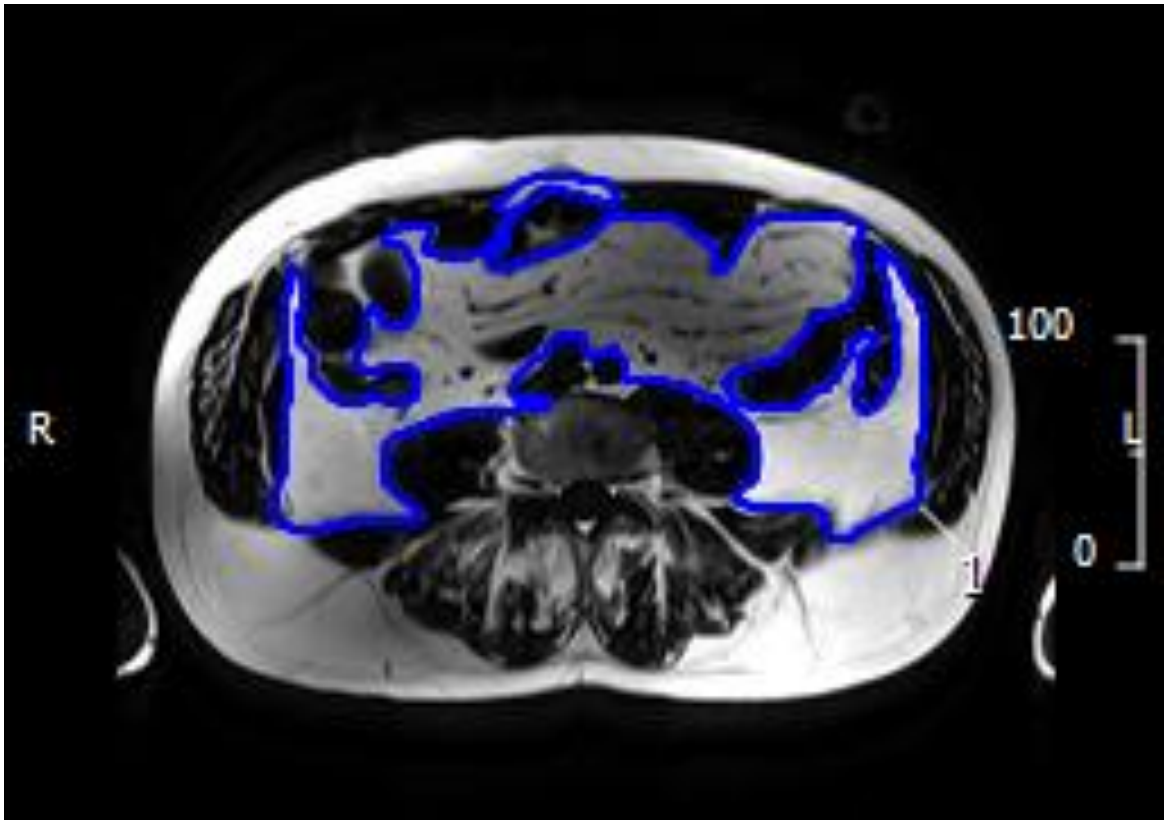


Figure 2.1: Transverse water-suppressed image at the level of L5, with abdominal visceral fat contoured in blue

6.1.2 Liver fat content

Hepatic fat was calculated using the Dixon method – in brief, to quantify fat, a 2d spoiled gradient recalled echo (SPGR) sequence was used with the following sequence parameters: flip angle 6°, TR/TE 55.5/1.25, 2.46, 3.69, 4.92, 6.15, 7.38, 8.61, 9.84ms; matrix size 128x104, slice thickness 6mm, bipolar gradient readout scheme. This was analysed using custom software (Liver MultiScan, Perspectum Diagnostics, Oxford, UK) which uses Iterative Decomposition of water and fat with Echo Asymmetric and Least-squares estimation (IDEAL)[180] to estimate fat and water fractions (figure 2.2).

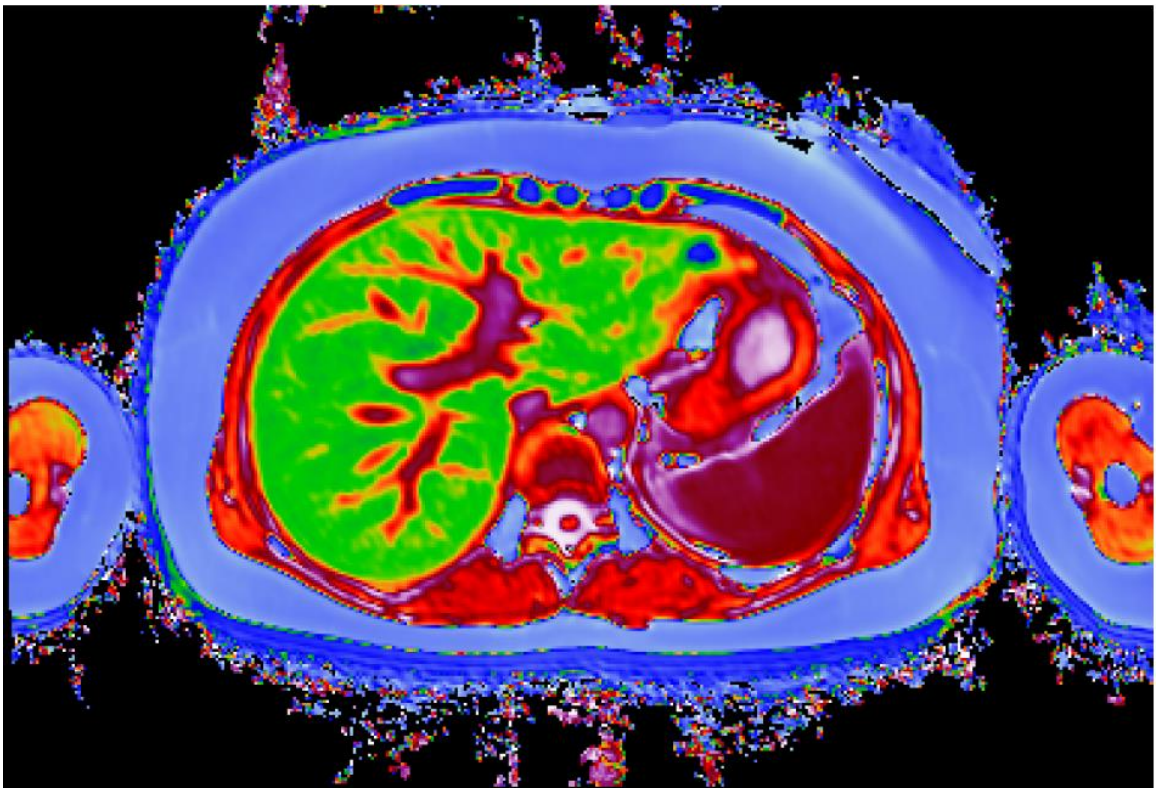


Figure 2.2: cT1 map at the level of the liver used for quantification of hepatic lipid content

6.1.3 Epicardial fat volume

Epicardial fat volume was measured by manual contouring of cardiac images, including only fat between the epicardial layer and internal visceral layer of the pericardium. The border between epicardial and pericardial fat was localised by visual inspection of the entire cine series in all slices. Areas of epicardial fat were traced manually on consecutive end-systolic short-axis images, beginning at the mitral valve and ending at the last slice containing cardiac adipose tissue. The areas obtained for each slice were summed together and multiplied by the slice thickness to yield epicardial fat volume. All epicardial fat analysis was performed by an experienced blinded observer (figure 2.3).

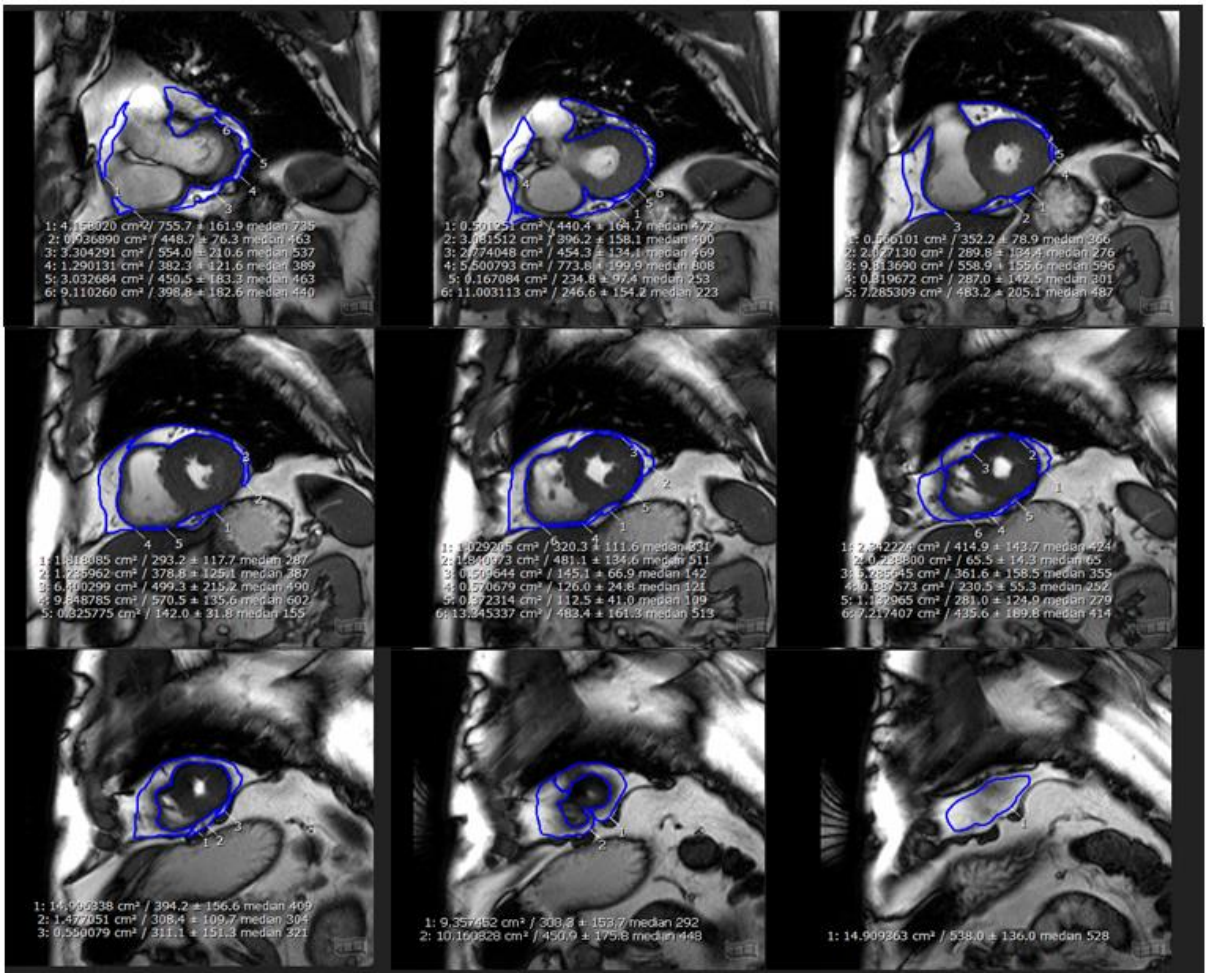


Figure 2.3: Contouring of epicardial fat volume on multiple short axis slices (outlined in blue)

6.2 Ventricular geometry and function

Cardiac imaging was acquired with steady-state free precession (SSFP) imaging. Images were acquired retrospectively cardiac-gated and acquired during end-expiratory breath-hold. Typical scan parameters were: voxel size 2.0x2.0x8.0mm, field of view (FOV) 400x400mm, TR/TE 39.6/1.12ms, flip angle 55°, matrix 192x192, Generalised AutoCalibrating Partial Parallel Acquisition (GRAPPA)=3, 24 reference lines, segments=15, concatenations=1. Following long axis pilots, horizontal long axis (HLA), vertical long axis (VLA) and a standard short-axis stack was performed.

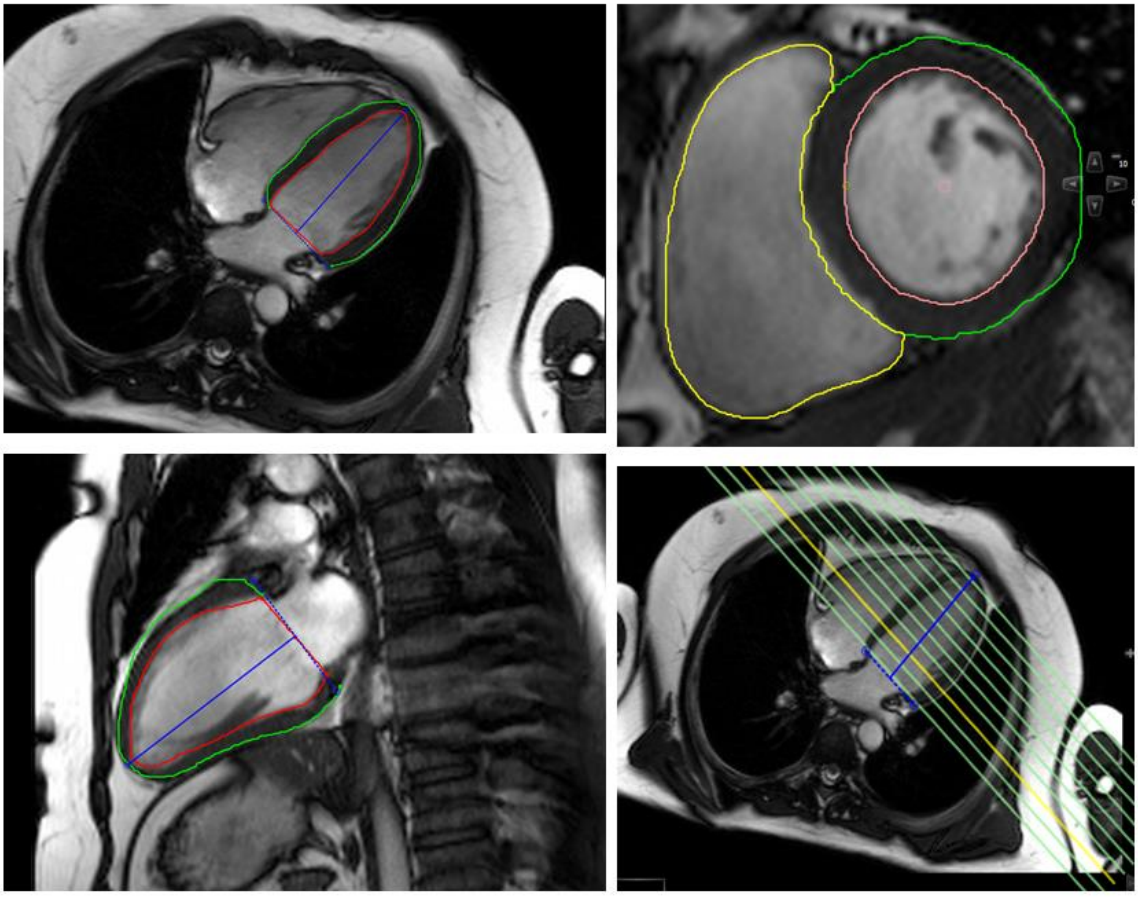


Figure 2.4: The acquisition and analysis of the horizontal long axis (upper left image), vertical long axis (lower left image), positioning of the short axis slices (lower right), and the short axis image (upper right)

LV mass, and biventricular volumes and ejection fraction were calculated from manual endocardial and epicardial contours (CVI42, Circle Cardiovascular Imaging Inc, Calgary, Canada) at end-systole and end-diastole (figure 2.5). Stroke volume was then calculated from end-diastolic volume (EDV) – end-systolic volume (ESV), and ejection fraction (EF) as (SV/EDV)*100. LV mass was then calculated as the endocardial volume subtracted from the epicardial volume measured at end-diastole, multiplied by myocardial specific gravity (1.05g/cm^3).

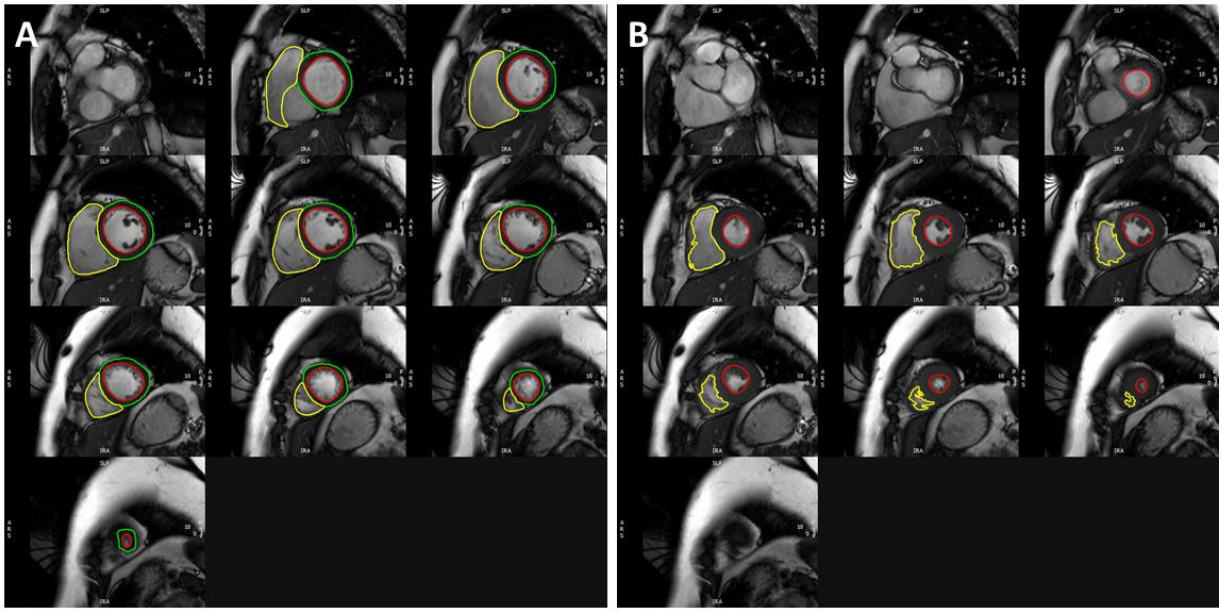


Figure 2.5: Endo- and epicardial contours manually drawn onto multiple short axis slices at end-diastole (A) and end-systole (B)

Information regarding left ventricular deformation was derived from feature tracking techniques, using the Tissue Tracking plugin in cvi42 (Circle Cardiovascular Imaging Inc, Calgary, Canada) to generate global measures of strain and strain rate. Figure 2.6 demonstrates a typical output from the software, demonstrating tracking of myocardial points based on endo- and epicardial contouring in both long and short axis planes, to generate polar plots, as well as x-y plots of (in this case) strain.

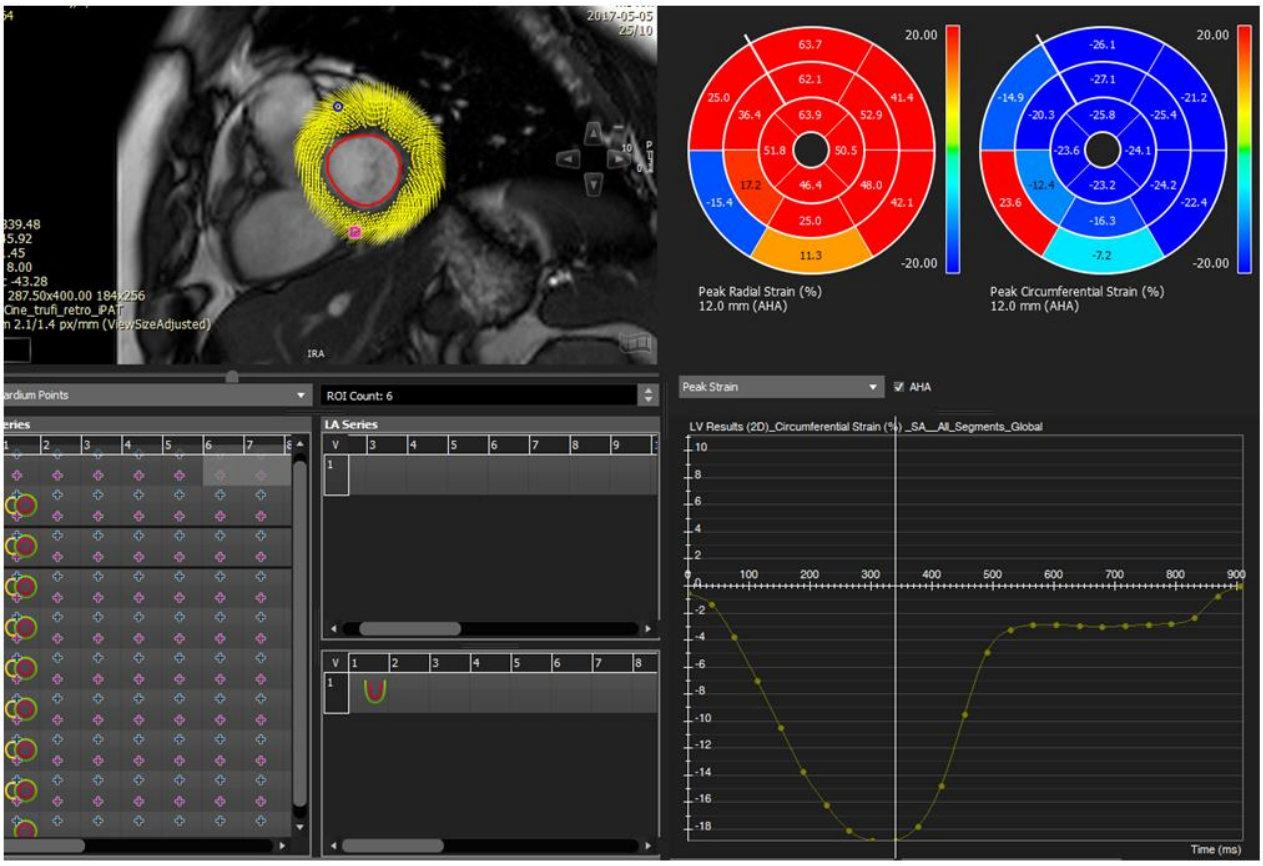


Figure 2.6: A typical output from feature tracking software

6.3 Aortic distensibility

Regional aortic distensibility (AD) was assessed using an SSFP cine sequence (thickness 7 mm), at 2 levels: 1) the ascending thoracic aorta (Ao), and 2) in the abdomen (DDA) 12 cm below this slice. During the acquisition of the images, a brachial blood pressure was recorded (Dinamap 1 846-SX, Critikon Corp).

The minimum and maximum aortic areas were contoured manually in cmr42 (figure 2.7), and aortic distensibility ($mmHg^{-1}$) was calculated as [(aortic area max – area min)/area min)/ pulse pressure] * 1000.

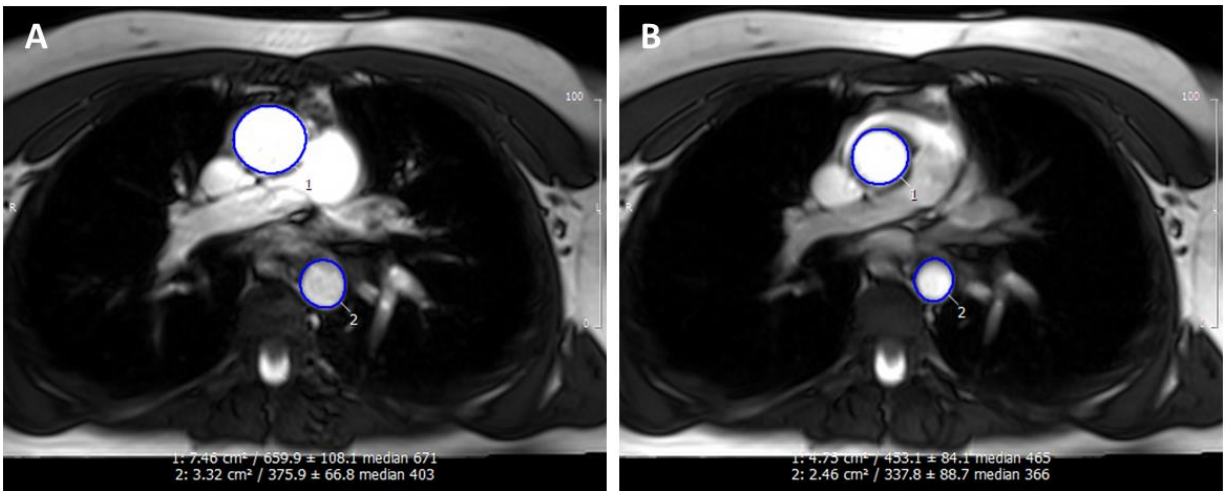


Figure 2.7: Contouring (in blue) of the ascending (labelled 1) and descending (labelled 2) aorta in systole (A) and diastole (B) to enable quantification of distensibility

6.4 ^{1}H-magnetic resonance spectroscopy

In order to quantify myocardial lipid content, myocardial ^{1}H-MR spectra were obtained from the inter-ventricular septum[181]. A conventional STEAM (Stimulated Echo Acquisition Mode) sequence was modified to achieve a short TE of 10ms, and water suppression was achieved using a WET (Water Elimination Transform) module. A calibration pulse sequence was implemented to identify the optimal water suppression pulse scaling factor. During the single-breath-hold acquisition, the modified STEAM sequence was repeated four times: a spectrum without water suppression was acquired first, with the frequency centred at the water frequency followed by three consecutive water-suppressed measurements with the frequency set to 3ppm. Also four non-averaged water spectra were acquired with a minimum TR of 4s in a separate breath-hold, setting the water suppression RF pulse power to zero. The total acquisition time was 5-7mins, including recovery time.

Results were analysed offline using in-house script within Matlab and the AMARES (Advanced Method for Accurate, Robust, and Efficient Spectral tracking) algorithm in the jMRUI software package.

Myocardial triglyceride content (MTGC) was calculated as a percentage relative to water: (signal amplitude of lipid/signal amplitude of water) ×100%[182].

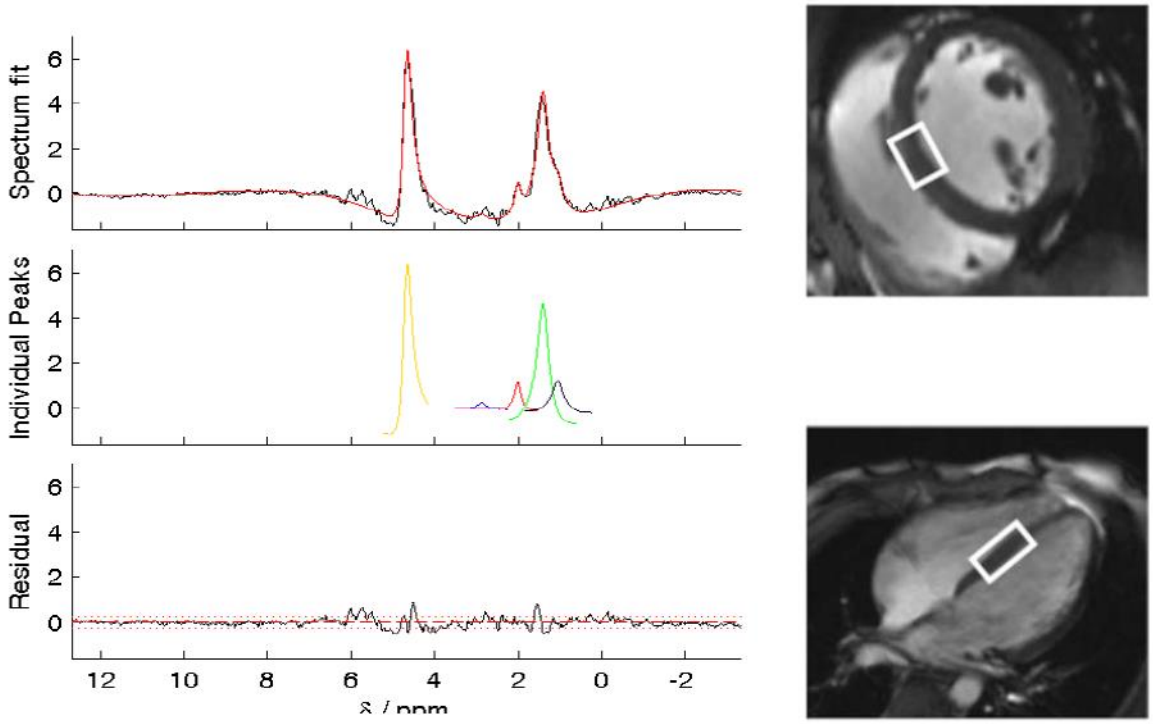


Figure 2.8: Representative ^{1}H spectroscopy from voxel in mid-septum (white box), indicating suppressed water peak (yellow) and lipid peak (green)

6.5 ^{31}Phosphorus magnetic resonance spectroscopy

^{31}P-MRS sequences were performed on the same 3T Trio Siemens scanner as described above, with a 10-cm loop transmit-receive ^{31}P surface coil (Pulse Teq, Chobham, UK). The ^{31}P coil was matched for each subject using a radio frequency sweeper (Morris Instruments Inc., Ottawa, Canada). Coil loading was measured by inversion-recovery on a fiducial fixed at the centre of the coil, and expressed as a reference voltage.

Creatine kinase k_f was measured using a 1D-CSI Triple Repetition Saturation Transfer (TRiST) sequence as developed by Schar et al[183]. This method measures the partially saturated magnetization $M_{z,PCr'}$ at short (TR_{short}) and long (TR_{long}) repetition periods in the presence of Delay Alternating with Nutation for Tailored Excitation (DANTE) saturation applied to the exchanging CK moiety, γ-ATP at -2.5ppm relative to PCr. $M_{0,PCr}$ is then measured fully-relaxed in the presence of control irradiation applied at +2.5ppm. T_1' is obtained from $M_z^{PCr'}$ (TR_{short}) and $M_z^{PCr'}$ (TR_{long}), according to the 2TR method. $M_0^{PCr'}$ is then calculated as $M_z^{PCr'}(TR_{short})/(1-\exp(-TR_{short} / T_1^{PCr'}))$.

The pseudo-first order forward rate constant (k_f) of creatine kinase is then calculated according to the equation:

$$k_f = \frac{1}{T_1'}\left(1 - \frac{M_0'}{M_0^{control}}\right)$$

The relative ratio of PCr to γ-ATP peaks in the fully relaxed spectrum is used to generate a PCr/ATP ratio, and the rate of ATP delivery, or CK flux, calculated by k_f *[PCr] where [PCr] is calculated by multiplying PCr/ATP by literature values for [ATP][93].

6.5.1 Dobutamine stress measurements

In order to measure the effect of catecholamine stress on k_f CK within a safe timeframe was previously only possible using the 'Two repetition time saturation transfer' (TwiST) method, published during the course of this study[184]. However, TwiST assumes PCr intrinsic T1 ($T1^*$) (ie the longitudinal relaxation constant of the nucleus not undergoing chemical exchange) to be constant; however, $T1^*$ in obesity is higher.

The average $T1^*$ was calculated, as per the equation:

$$T_1^* = T_1' \left(\begin{matrix} M_{PCr}^{control} \\ M_{PCr}' \end{matrix} \right)$$

T1* for PCr was 5.69±1.43s for obese and 4.67±1.92s for normal weight (p=0.02)[185]. This suggests that to accurately measure CK k_f at stress, T1* must be determined per subject, giving rise to the novel 'StreST' protocol (figure 2.8), and allowing measurements to be completed within 18 minutes.

Dobutamine was infused via a peripheral venous cannula, with continuous ECG and NIBP monitoring, at an initial dose of 5mcg/kg/min, with dose increasing every 3 minutes to 10, 20, 30 and 40 mcg/kg/min, as necessary to achieve a target heart rate of 65% maximum (maximum heart rate calculated as 220-age). Long axis cine images were repeated at maximum stress to assess contractile response to stress, as well as exclude any regional wall motion abnormalities.

^{31}P-MRS data was analysed post hoc using AMARES analysis software within Matlab, and an unadjusted PCr/ATP generated for rest and stress, as well as the creatine kinase k_f.

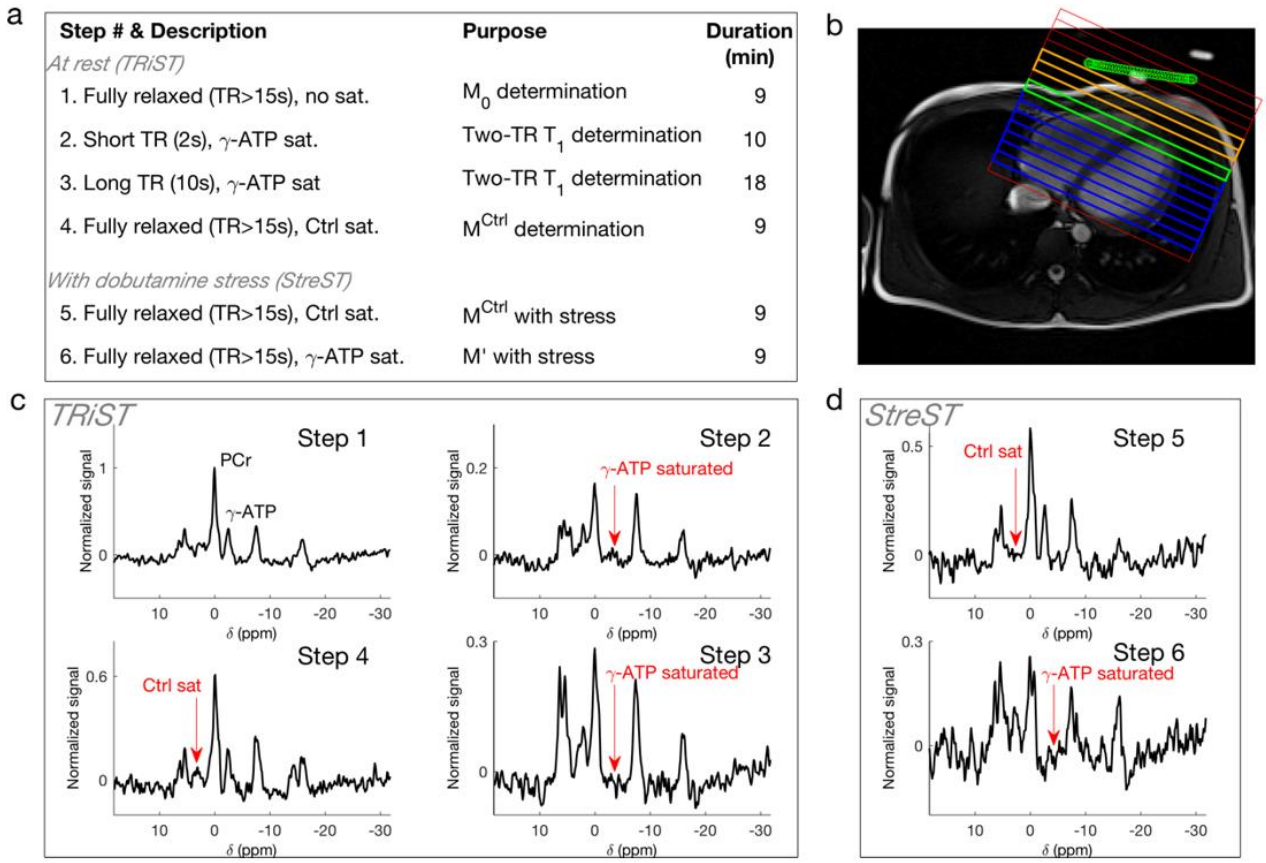


Figure 2.8: a: Six-step StreST protocol (including TRiST as steps 1–4). B: H localiser with 1D CSI grid overlaid. The coil position is the most anterior box marked in green. Orange = Skeletal muscle, blue = myocardium, green = most anterior myocardial voxel (coil voxel distance < 60 mm). c: Spectra acquired from the 4 steps of TRiST on a healthy, normal-weight subject. D: Spectra acquired during dobutamine stress as part of the extension StreST measurement (Clarke et al[185]).

7. Echocardiography

Standard transthoracic echocardiography was performed on a Philips Epiq system (Philips, Netherlands) in the left lateral position with ECG gating, to determine diastolic function; pulse wave velocity was measured at mitral valve inflow to calculate E/A ratio, and tissue Doppler at lateral and medial mitral valve annulus to generate E/e' ratios, as well as the mean of medial and lateral measurements.

8. Exercise testing

8.1 Cardiopulmonary exercise testing

All participants underwent maximal exercise testing on a cycle ergometer according to an individualised ramped protocol. Continuous recording of respiratory gas exchange parameters was taken during the test and recovery (Cortex Metalyser). Participants were asked to exercise until maximal exertion. Gas exchange data was acquired breath by breath and averaged over 20-second intervals. Peak $\dot{V}O_2$, heart rate and respiratory exchange ratio were recorded, as well as $VE/\dot{V}CO_2$ slope. Heart rate reserve was calculated as increase in heart rate (HR) with exercise divided by difference between resting HR and age-predicted maximum HR[186].

Unfortunately, pulmonary function tests were not routinely acquired during CPEX testing – in hindsight, we now appreciate more about the abnormalities in respiratory function which result from both obesity and heart failure, and the absence of this data is a limitation.

8.2 Six minute walk test

All participants underwent a standardised walking test[187] in which they were instructed to walk at a brisk pace back and forth along a 35m corridor for six minutes. The total distance (m) achieved was recorded.

9. Dietary intervention

Where relevant, participants were asked to adhere to a dietary intervention before repetition of the study investigations. This was either a low glycaemic index, calorie controlled diet, or a Very Low Calorie Diet.

9.1 Low Glycaemic Index diet

This commercially available diet programme is both calorie controlled with a focus on fresh produce, and easy to understand and follow. The glycaemic index principle[188] focuses on differentiating between carbohydrates based on the rapidity and degree to which they cause blood glucose to rise. High glycaemic index foods such as simple sugars or white bread cause a dramatic rise in blood glucose, whereas lower glycaemic index foods such as wholemeal bread and boiled potatoes, lead to a slower change. There is significant evidence suggesting that LGI diets improve insulin resistance in diabetics[189] and overweight/obese[190], as well as protecting against coronary disease[191].

Volunteers were supplied with a copy of the diet programme[192], and asked to adhere for a period of 12 months. Support was available in person, over the telephone or by email as instigated by the participant.

9.2 Very Low Calorie Diet

The Very Low Calorie Diet (VLCD) has experienced a recent resurgence in popularity, due to the impressive results of the DiRECT trial which demonstrated that the programme was effective in reversing diabetes[193]. The diets comprise nutritionally complete meal replacement products, usually soups and shakes, to be taken in place of solid food for the period of the dietary intervention.

Volunteers followed a supervised Very Low Calorie Diet (800kcal per day; 59% carbohydrate, 26% protein, 13% fat, 3% fibre; replacement diet programme, Counterweight Ltd, Glasgow, UK) for 8 weeks before gradual meal reintroduction. Antihypertensive therapy was discontinued at the start of the diet intervention. Volunteers were advised to continue current levels of physical activity, and increase fluid intake with water or calorie free fluids to ensure adequate hydration.

10. Blood collection, processing, storage and analysis

20ml of venous blood was drawn at the beginning of all study visits. Samples were sent to the Oxford University Hospitals NHS Foundation Trust laboratory for measurement of haematocrit, liver function tests, fasting lipid profile, fasting glucose and insulin, and beta-natriuretic peptide according to standard clinical protocols.

Additionally, 3 EDTA tubes were processed per participant, centrifuged at 4000rpm at 4°C for 5 minutes. The remaining serum was stored at -80°C until batch analysis at study completion. The following assays were performed where relevant:

10.1 Free fatty acids

FFAs were analysed using commercially available colorimetric assay kits (Cayman Chemical, USA; coefficient of variability<6%).

10.2 β-hydroxybutyrate

β-hydroxybutyrate was measured using a commercially available colorimetric assay kit (Cayman Chemical, USA; CV<4%).

10.3 Leptin

Leptin was measured using a commercially available ELISA kit (Sigma-Aldrich, St Louis, Missouri, USA; intra-assay coefficient of variability<12%).

10.4 Adiponectin

Adiponectin was measured using a commercially available ELISA kit (Sigma-Aldrich, St Louis, Missouri, USA; intra-assay coefficient of variability<12%).

11. Statistical analysis

All statistical analysis was performed using commercial software (SPSS 23, Chicago).

An *a priori* power calculation to determine cohort size in the weight loss intervention study, was performed based on previously published changes in EDV[58]. Assuming normal distribution and paired t-test analysis (α 0.05, power 0.8, correlation 0.7), at least 16 participants were required to successfully lose weight to show a 9±6% fall in EDV over a similar timeframe. Assuming a 25% drop out rate for device implantation and a 70% success rate in losing weight, at least 32 participants in the HFrEF group would be needed.

All data is presented as mean ± standard deviation, unless stated otherwise. Normality was assessed using a Shapiro-Wilk test to determine use of parametric or non-parametric tests. Homogeneity of distribution was assessed using Levene's test, before determination of statistical significance, either by Chi-squared or McNemar's tests in the case of categorical data, or ANOVA or student's t tests for continuous data (paired or independent where relevant). Where a single measurement was repeated three or more

times, repeated measured ANOVA was used. Statistical significant between groups of non-parametric data was determined with the Kruskal Wallis or Mann Whitney U test.

Pearson's or Spearman's correlation models were used to determine association. Choice of regression model was determined through analysis of the simplest relationship which obtained the best R^2 – in most cases, this was a simple linear regression, and on one occasion (Chapter 3) a semi-log regression model.

Values of $p<0.05$ were considered as statistically significant.

Chapter Three

The Cardiovascular Impact of a Very Low Calorie Diet

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1. Background

Given the link between obesity and development of cardiovascular disease, exploring options for the management of obesity, preventing its complications, and their impact on the cardiovascular system is of general importance, and particular relevance for this thesis. The use of Very Low Calorie Diets (VLCDs) has recently seen a resurgence in popularity for the treatment of obesity, with 6-800kcal per day of meal replacement products proving effective and straightforward to follow. These programmes may be of particular relevance for cardiologists, as they have recently been demonstrated to be highly effective, reversing whole body insulin resistance and diabetes in the majority of participants in the recent DiRECT trial[193].

Diabetes and obesity have been linked to myocardial dysfunction due to the combination of peripheral and myocardial insulin resistance, high circulating fatty acids (FAs) leading to myocardial steatosis, and subsequent metabolic inefficiency and inflexibility[194]. This metabolic dysfunction is linked to depletion of energetic stores[25], and, as diastole is inherently more sensitive to ATP shortage than systole due to the high energy demands of SERCA ATPase, diastolic dysfunction may represent the first manifestation of metabolic dysfunction in the myocardium.

Additionally, hormones secreted by adipose tissue, such as leptin and adiponectin, have been found to have significant effects on the intracellular signalling cascades associated with substrate selection in the myocardium[111, 195], and the impact of sudden body fat reduction on the concentrations of these hormones is unknown.

Previous studies have demonstrated that weight loss is associated with reversal of abnormalities in substrate utilisation, and also diastolic dysfunction[85]. Hence manipulating myocardial substrate selection with a dietary intervention is a potential mechanism for preventing and/or treating diastolic dysfunction, in addition to its obvious benefits in terms of body fat reduction.

Whilst the combination of improved insulin sensitivity and weight loss that occur during VLCDs should theoretically be beneficial to the cardiovascular system[58], this may well not be the case. Severe caloric restriction elevates circulating FA levels due to the rapid mobilisation of fat stores. Acute elevation of FA levels has been shown to impair vascular function in humans[196], and lead to myocardial lipid accumulation and dysfunction in animals[197, 198], with a 3 day extreme VLCD (471kcal/day) in humans also resulting in impaired diastolic function[199, 200].

The effects of the now widely used modern VLCDs on myocardial and vascular function in a real-world setting are yet to be established, but are an important clinical consideration given both the historical context of increased cardiovascular risk with VLCDs[201], and the burgeoning worldwide prevalence of heart failure associated with obesity. They are also an interesting experiment in examining the impact of acute substrate manipulation on the cardiovascular system, so are particularly pertinent to this thesis.

With these questions in mind, I sought to investigate, in a healthy obese population, whether any changes in cardiovascular metabolism, energetics and function occur over the course of a modern 8-week VLCD regime, exploring these changes alongside the expected peripheral metabolic improvements and changes in adipokine profile, and demonstrate whether these dietary interventions have potential for treatment of obesity-related cardiac dysfunction. In addition, volunteers were assessed at an early time-point (1 week after the initiation of the diet), in order to investigate the acute metabolic effects in the absence of significant changes in body fat.

2. Methods

For detailed methods, see Chapter Two.

In brief, the local ethics committee approved the study (REC SC/15/004), and informed written consent was obtained from each patient. 40 volunteers (25 following VLCD, 15 controls) were recruited from the Oxfordshire population by poster advertisement. Inclusion criteria were age>18 years, BMI>30 kg/m^2 (VLCD group) or <25kg/m^2 (control). Exclusion criteria were insulin therapy for diabetes, current or previous history of coronary disease, valvular heart disease, any clinical history or imaging evidence of systolic heart failure (eg LVEF <57% by MR assessment (the local lower limit of normal in a tertiary referral CMR centre) or impaired LV function on echo), pregnancy or planned pregnancy, and other standard contraindications to magnetic resonance (MR) scanning as described in Chapter Two.

Participants underwent the following investigations at baseline, at 1 and 8 weeks after commencing the VLCD in those allocated to the dietary intervention.

2.1 Anthropomorphic and metabolic assessment

Body composition was analysed by bio-impedance assessment. A standard 12-lead electrocardiogram was recorded, and non-invasive blood pressure measured. Fasting venous blood was drawn to measure circulating biomarkers: glucose, cholesterol, triglycerides, BNP as an indirect indicator of ventricular function, FFA, β-hydroxybutyrate, adiponectin and leptin.

In addition at baseline and 8 weeks, a mixed meal tolerance test was performed (37g carbohydrate, 12g protein, and 12g fat (200ml Fortisip drink, Nutricia, Schiphol, The

Netherlands)), with venous glucose and insulin drawn additionally at 30, 60 and 120 minutes. Insulin resistance was calculated both using the HOMA-IR model at baseline, 1 week and 8 weeks ((fasting glucose x fasting insulin)/22.5), and the Matsuda index at baseline and 8 weeks (10000/√ ((fasting glucose x fasting insulin) x (mean glucose x mean insulin during meal tolerance test)).

2.2 Magnetic resonance imaging

Left ventricular geometry and systolic function, liver fat, myocardial triglyceride content (MTGC), myocardial energetics (PCr/ATP, baseline and 8 weeks only), regional aortic elastic function, epicardial and abdominal visceral fat were all assessed using a combination of multiparametric magnetic resonance imaging and multinuclear spectroscopy (3 Tesla Trio, Siemens, Erlangen, Germany) as described in Chapter Two, and below where techniques varied.

2.2.1 Myocardial energetics

Myocardial PCr/ATP was derived from a cardiac-gated fully relaxed (T_R>15sec) 1D-CSI acquisition using a 10 cm loop transmit-receive ^{31}P surface coil (Pulse Teq, Chobham, UK). The ^{31}P coil was matched for each subject using a radio frequency sweeper (Morris Instruments Inc., Ottawa, Canada). In-house software within Matlab was used for analysis.

2.3 Echocardiography

Echocardiography was performed on a Philips Epiq system, to determine diastolic function; pulse wave velocity was measured at mitral valve inflow and tissue Doppler at lateral and medial mitral valve annulus.

2.4 Very Low Calorie Diet

Volunteers followed a supervised Very Low Calorie Diet (800kcal per day; 59% carbohydrate, 26% protein, 13% fat, 3% fibre; replacement diet programme, Counterweight Ltd, Glasgow, UK) for 8 weeks before gradual meal reintroduction. Antihypertensive therapy was discontinued at the start of the diet intervention. Volunteers were advised to continue current levels of physical activity, and increase fluid intake with water or calorie free fluids to ensure adequate hydration.

2.5 Statistics

Statistical analysis was performed using commercial software (SPSS, Chicago). All data is presented as mean ± standard deviation. Normality of distribution was confirmed before determination of statistical significance, either by Chi-squared tests in the case of nominal data, student's t tests for continuous data (independent for comparison of baseline characteristics), or repeated measures ANOVA (for comparison within individuals at the 3 time points) with the Greenhouse-Geisser correction, with post-hoc tests using the Bonferroni correction. Non-parametric data was analysed with the Mann Whitney U test, or Kruskal Wallis test, with paired data using the Wilcoxon Rank test. Pearson's correlation models were used where relevant. The distribution of adiponectin concentrations was negatively skewed, and logarithmic transformation performed prior to analysis. Values of $p<0.05$ were considered as statistically significant.

3. Results

Of the 25 volunteers, the majority were female (17/25). 4 were taking antihypertensive medication, 3 cholesterol-lowering medication and 2 taking oral hypoglycaemic medication for diabetes (Table 3.1). 2 volunteers withdrew by 1 week, and a further 2 by 8 weeks – there were no significant differences in characteristics in these individuals to those who completed the study.

3.1 Baseline

3.1.1 Anthropometric and metabolic characteristics

VLCD volunteers and controls were well matched for age (study group 49±14 years, controls 45±17 years, p=0.410) and sex (study group 32% male, controls 40% male, p=0.717). As expected, the average BMI of the obese volunteers was significantly higher than controls (36.8±5.8 vs 24.0±2.4 kg/m^2, p<0.001) with higher total fat mass (by 29kg) and nearly 3-fold greater visceral fat (both p<0.001). Liver fat was also significantly higher (VLCD 7.4±6.3% vs 0.9±0.6% p<0.001), and above the threshold for diagnosis of non-alcoholic fatty liver disease (>5%) in 15 of the 25 obese individuals. Epicardial fat volumes were also higher (102±33 vs 38±15cm^3, p<0.001). On average, blood pressure in the study group was systolic 135±20mmHg, and diastolic 89±11 mmHg, with both values significantly higher than controls (p<0.01, Table 3.1). Additionally, heart rate was higher in the obese study group (67±14bpm compared to 58±8bpm in the normal weight controls, p=0.039).

Table 3.1: Baseline anthropomorphic parameters for controls and study population

	Normal controls n=15	Study population n=25	p
Body mass index (kg/m^2)	24.0±2.4	36.8±5.8	**<0.001**
Age (years)	45±17	49±14	0.410
Male (%)	6 (40)	8 (32)	0.717
Systolic blood pressure (mmHg)	121±15	136±19	**0.008**
Diastolic blood pressure (mmHg)	77±11	89±11	**0.004**
Resting heart rate (bpm)	58±8	67±14	**0.039**
Metabolic status:			
Total cholesterol (mmol/l)	4.6±1.0	5.4±1.0	**0.017**
Triglycerides (mmol/l)	1.0±0.5	1.6±0.7	**0.003**
Glucose (mmol/l)	4.8±0.3	5.4±0.9	**0.011**
Insulin (mmol/l)	35±13	94±48	**0.019**
HOMA-IR	1.2±0.5	4.3±5.1	**0.029**
Leptin (ng/ml)	23±12	63±33	**<0.001**
Adiponectin (µg/ml)	19±20	41±50	0.092
BNP (pg/ml)	6±4	5±3	0.311
Body fat distribution:			
Body fat mass (kg)	16±7	45±13	**<0.001**
Body fat percentage (%)	23±11	43±8	**<0.001**
Abdominal visceral fat area (cm^2)	56±45	162±81	**<0.001**
Liver fat (%)	0.9±0.6	7.4±6.3	**0.001**
Epicardial fat (cm^3)	38±15	102±33	**<0.001**
Myocardial triglyceride content (%)	1.0±0.7	2.2±1.1	**0.003**

Although fasting glucose (5.4±0.9 mmol/l), total cholesterol (5.4±1.0 mmol/l) and triglycerides (1.6±0.7 mmol/l) were within the normal range, they were all higher than controls (all $p<0.05$, Table 3.1). The obese VLCD group was significantly insulin resistant, whereas controls were more insulin sensitive (HOMA-IR 4.3±5.1, vs 1.2±0.5, $p=0.029$).

Leptin concentrations were significantly higher in the VLCD group compared to controls (63±33ng/ml vs 23±12ng/ml, $p<0.001$). Leptin concentration correlated with fat mass (r 0.719, $p<0.001$), although interestingly not with LV mass ($p=0.205$). Although there was no significant difference between adiponectin concentrations between the two groups, there is a numerical trend towards higher values in the obese group (41±50 μg/ml compared to 19±20 μg/ml, $p=0.092$). This may reflect the degree of adiponectin resistance which is known to occur in the presence of insulin resistance.

3.1.2 Baseline cardiac function and metabolism

In agreement with previous studies, the obese VLCD group had higher LV mass (by 16g, $p=0.046$; table 3.2), two-fold higher myocardial triglyceride levels ($p=0.003$) and reduced myocardial energetics (PCr/ATP 1.9±0.3 compared to 2.2±0.2, $p=0.003$). Importantly, circulating fatty acid levels correlated with MTGC at baseline ($r=0.479$, $p=0.028$, fig 3.1).

Table 3.2: Baseline cardiac phenotype in controls and study population

	Normal controls n=15	Obese study population n=25	p
LV geometry:			
LV end-diastolic volume (ml)	146±26	156±27	0.267
LV mass (g)	98±20	114±27	**0.046**
LV mass-to-volume ratio	0.66±0.06	0.74±0.15	0.056
LV systolic function:			
LV ejection fraction (%)	65±7	66±5	0.408
Peak radial strain (%)	42±8	49±6	**0.011**
Peak circumferential strain (%)	-20±2	-20±2	0.275
Peak longitudinal strain (%)	-16±2	-16±2	0.831
LV diastolic function:			
LV E/e'	7.6±1.3	8.6±1.4	**0.022**
Peak radial diastolic strain rate (1/s)	-3.7±1.1	-2.7±1.5	**0.020**
Aortic distensibility ($mmHg^{-1}$):			
Ascending aorta	4.1±2.7	5.4±3.8	0.284
Distal descending aorta	7.3±2.4	4.9±3.8	**0.026**

Although global systolic function was normal, and not different to controls (LVEF 66% vs 65% p=0.400), there was evidence of subclinical changes in systolic function with increased peak systolic radial strain (by 14%, p=0.011); despite this, there was no significant difference in other measured strain parameters. Diastolic function was significantly impaired in the obese VLCD group at baseline (average E/e' 8.4±1.4 vs 7.6±1.3, p=0.022).

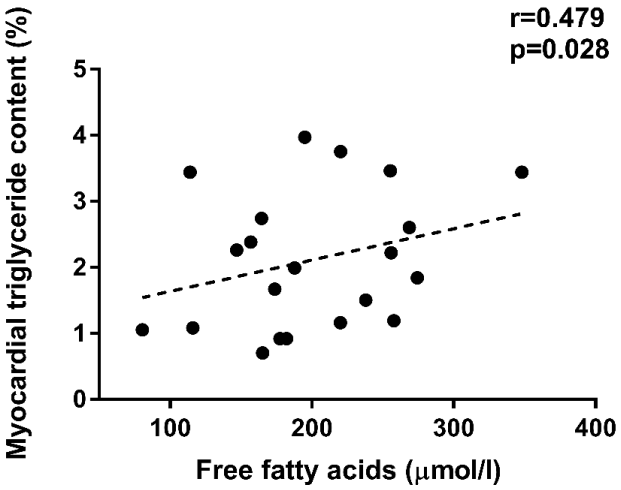


Figure 3.1: Circulating levels of free fatty acids correlate with myocardial triglyceride content

3.1.3 Aortic elastic function

In line with previous studies, although aortic distensibility was similar to controls in the ascending aorta (p=0.28), abdominal aortic elastic function was significantly impaired (by 33%) in the obese VLCD group prior to dietary intervention (4.9±3.8mmHg^{-1} vs 7.3±2.4 mmHg^{-1}, p=0.026).

3.2 The effects of 1 week of VLCD

Following 7 days of VLCD, there were small but significant reductions in BMI (1.3±0.5kg/m^2), total fat mass (2.4±1.6kg), visceral (by 14±24%) and epicardial fat (by 10±18% all p<0.05, Table 3.3).

Table 3.3: The metabolic effects of a Very Low Calorie Diet after 1 and 8 weeks

	Baseline n=25	1 week n=23	8 weeks n=21
BMI (kg/m^2)	36.8±5.8	35.1±5.8**	32.3±5.8** ‡
Body fat mass (kg)	45±13	42±14**	35±14** ‡
Body fat percentage (%)	43±8	40±9*	36±10** ‡
Systolic blood pressure (mmHg)	136±19	128±14*	124±12*
Diastolic blood pressure (mmHg)	89±11	87±16	80±10
Resting heart rate (bpm)	69±13	62±10**	59±7*
Metabolic parameters:			
Total cholesterol (mmol/l)	5.4±1.0	4.5±1.0**	4.6±1.0**
Triglycerides (mmol/l)	1.6±0.7	1.2±0.6**	1.4±0.9
Beta-hydroxybutyrate (mmol/l)	0.25±0.10	0.55±0.35**	0.46±0.23**
Free fatty acids (µmol/l)	197±71	284±78**	314±143**
BNP (pg/ml)	4.8±2.8	3.7±1.7	5.5±3.0*
Glucose (mmol/l)	5.4±0.9	4.7±0.9**	4.8±0.6**
HOMA-IR	4.3±5.1	1.7±1.4*	1.3±0.7*
Matsuda index	19±13	-	24±12**
Leptin (ng/ml)	61±35	-	26±17**
Adiponectin (µg/ml)	41±50	-	38±67
Body fat distribution:			
Liver fat (%)	7.4±6.3	5.5±5.0**	3.3±3.0* †
Myocardial triglyceride content (%)	2.2±1.1	3.1±1.7*	1.5±0.6* †
Epicardial fat (cm^3)	102±33	91±27*	86±29** ‡
Abdominal visceral fat area (cm^2)	162±81	153±93	102±53** ‡

* indicates $p<0.05$ compared to baseline, ** indicates $p<0.01$ compared to baseline

Week 8 data only: † indicates $p<0.05$ compared to normal controls, ‡ indicates $p<0.01$ compared to normal controls

Despite this modest weight loss, a relatively larger fall in hepatic fat occurred (31±24%, p<0.001; figure 3.2A) and was associated with significant improvements in fasting glucose (by 13±14%), and even larger improvements in insulin sensitivity (HOMA-IR, by 42±26%, all p<0.01, table 3.3). Interestingly, the change in liver fat content did not correlated with the improvement in insulin resistance. Total cholesterol was also lower at one week (by 18±5%). In addition to metabolic improvements, and despite the withdrawal of antihypertensive medication, systolic blood pressure fell (by 6±9mmHg to 128±14mmHg, p=0.035), as did resting heart rate (from 69±13bpm to 62±10bpm, p=0.003).

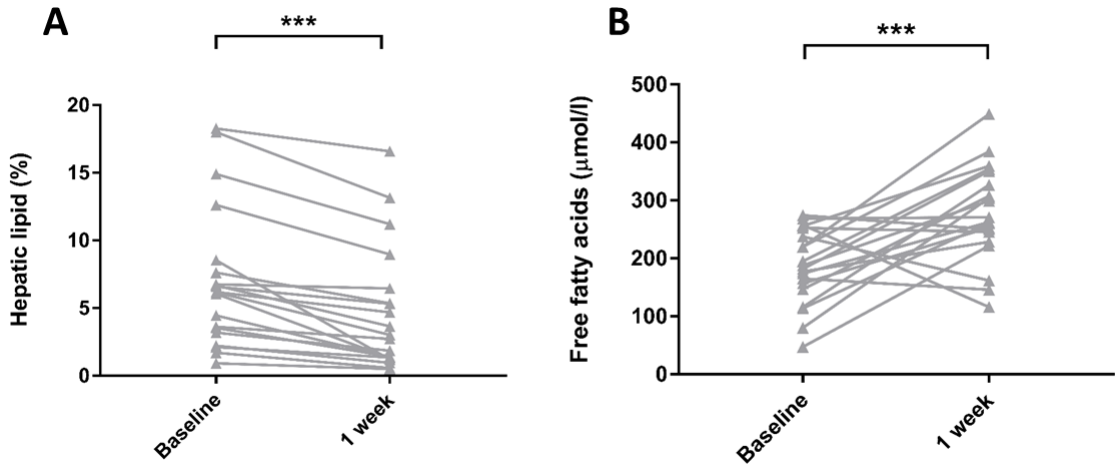


*Figure 3.2: Hepatic lipid (A), and free fatty acids (B) at baseline and 1 weeks after initiation of the Very Low Calorie Diet (*** indicates p<0.001)*

Consistent with a period of severe caloric restriction, and demonstrating adherence to the diet protocol, blood β-hydroxybutyrate levels increased (from 0.25±0.10mmol/l to 0.55±0.35mmol/l, p=0.002) and circulating levels of fatty acids rose by 55% during the first week of VLCD (p=0.017, fig 3.2B).

In contrast to other recorded fat depots, myocardial triglyceride content increased by 50% (from 2.1±1.1% to 3.1±1.7%, p=0.030, figure 3.3A).

At this time-point, there was a demonstrable deterioration in both LV systolic function (LVEF 66±5% to 62±6%, p=0.041), and diastolic function (average E/e' 8.6±1.4 to 9.4±1.7, p=0.019, figure 3.3B).

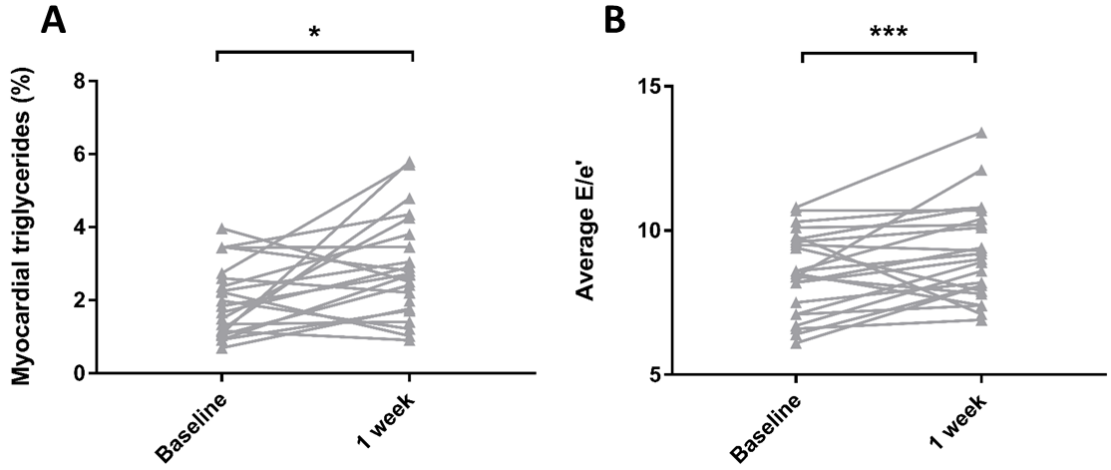


*Figure 3.3: Myocardial triglyceride content and left ventricular diastolic function at baseline and after 1 week on a Very Low Calorie Diet. * indicates p<0.05, *** p<0.001*

There was a relationship between the change in MTGC and the change in both systolic (LVEF, r=-0.376, p=0.112) and diastolic function when measured with peak diastolic strain rate (PDSR, r=0.458, p=0.056) after one week, albeit not statistically significant.

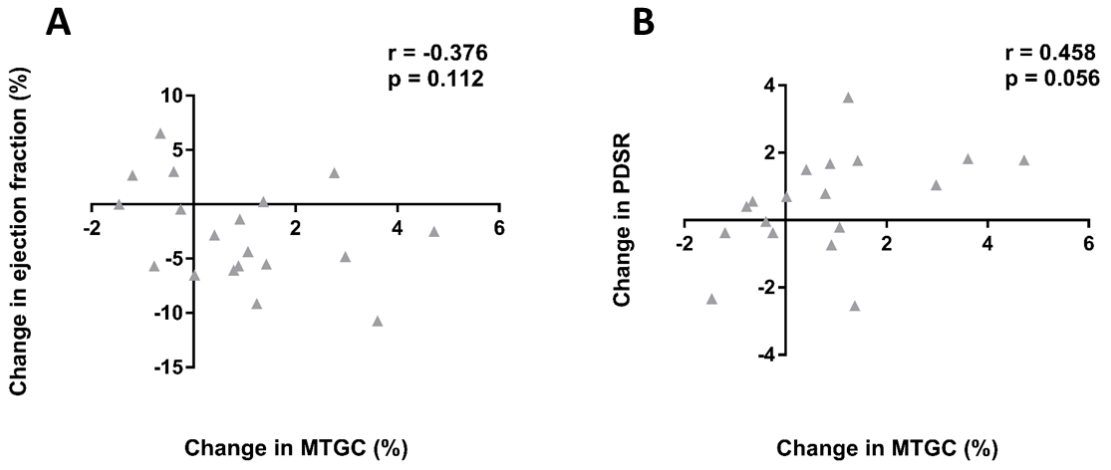


Figure 3.4: The relationship between change in myocardial triglyceride content, and the change in left ventricular systolic (A) and diastolic (B) function

There was a correlation between change in circulating β-hydroxybutyrate and the degree of systolic impairment at 1 week (figure 3.5), suggesting that the deterioration in systolic function was less pronounced in those individuals with higher levels of circulating ketone bodies.

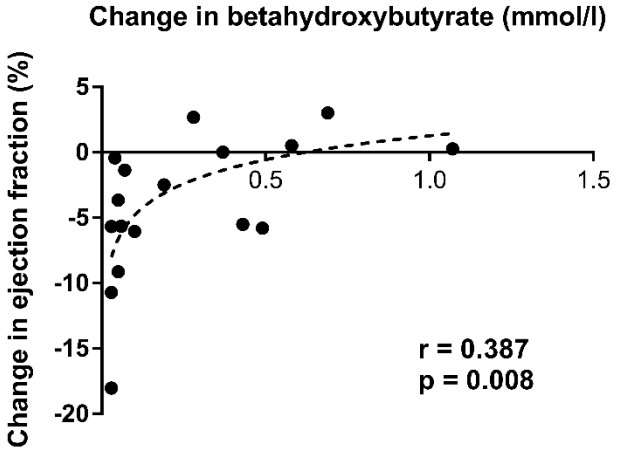


Figure 3.5: The deterioration in systolic function is greater when circulating ketones are lower (dotted line indicates semi-log regression curve).

Interestingly, BNP levels remained low and unchanged during this initial period (4.8±2.8 vs 3.7±1.7 pg/ml, Table 3.3), suggesting that either the functional changes measured were insufficient to increase levels of BNP secretion, or that the concomitant fall in body fat mass cancelled out any observed change.

In addition, there were significant changes in LV geometry, with LVEDV being smaller after 1 week of VLCD (by 13ml, $p<0.05$, table 3.4). In support of the absence of changes in circulating volume driving these changes, blood haematocrit did not change from baseline (0.422±0.038 compared to 0.428±0.38, $p=0.216$).

Furthermore, aortic elastic function was reduced from baseline both in the ascending (4.3±3.4mmHg^{-1} from 5.4±3.8mmHg$^{-1,}$ $p=0.009$) and descending aorta (3.8±0.9mmHg^{-1} from 4.1±2.5mmHg^{-1}; $p=0.086$).

In summary, after one week of VLCD, despite significant metabolic benefits with large reductions in liver fat and improvements in insulin sensitivity, the already impaired cardiovascular system in obesity deteriorated further, with elevation in circulating FFAs and myocardial triglyceride content, reduced left ventricular systolic and diastolic function, and further increase in aortic stiffness.

3.3 The effect of eight weeks of VLCD

After 8 weeks (n=21, average timeframe 53±13 days; tables 3.3 and 3.4), total body weight continued to fall (by 12% from baseline, $p<0.01$), with total body fat mass falling by 23% from baseline ($p<0.001$). Visceral fat fell to a greater extent than total body fat over the study period (by 37%). Liver fat continued to fall (3.3±3% from 5.5±5%, $p<0.05$, figure 3.6A).

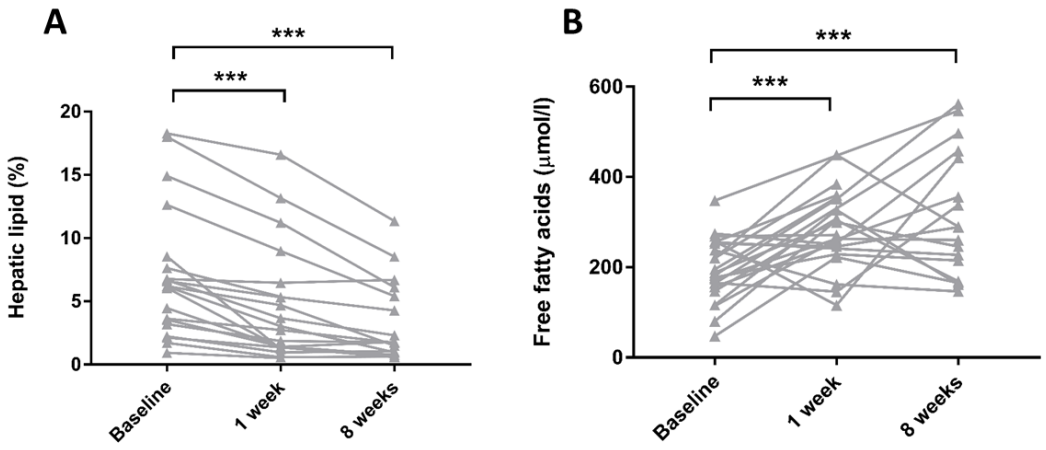


*Figure 3.6: Hepatic lipid and free fatty acids at 8 weeks compared to baseline and 1 week. *** indicates p<0.001.*

Table 3.4: The cardiovascular effects of a Very Low Calorie Diet after 1 and 8 weeks

	Baseline n=25	1 week n=23	8 weeks n=21
LV geometry and function:			
LV end-diastolic volume (ml)	156±27	144±26**	151±27*
LV ejection fraction (%)	66±5	62±6*	64±5
LV mass (g)	114±27	110±23	107±23*
LV strain analysis:			
Peak radial strain (%)	49±2	42±3	45±2
Peak circumferential strain (%)	-20±2	-19±2**	-19±2
Peak longitudinal strain (%)	-16±1	-15±2	-15±3
Peak radial diastolic strain rate (1/s)	-2.7±1.0	-3.2±0.8	-3.2±1.0*
LV average E/e'	8.6±1.4	9.4±1.7**	7.5±1.5**
Myocardial PCr/ATP	1.9±0.3	-	2.2±0.2*
Aortic distensibility ($mmHg^{-1}$):			
Ascending aorta	5.4±3.8	4.3±3.4**	5.8±4.6
Distal descending aorta	4.1±2.5	3.9±2.1	5.0±2.8

* indicates $p<0.05$ compared to baseline, ** indicates $p<0.01$ compared to baseline

Week 8 data only: † indicates $p<0.05$ compared to normal controls, ‡ indicates $p<0.01$ compared to normal controls

Similarly, total cholesterol, triglycerides and fasting glucose continued to fall, and by the end of the dietary period, despite BMI remaining in the obese range (32.3±5.8kg/m^2), levels were not significantly different from normal controls (table 3.3). Insulin sensitivity continued to improve between 1 and 8 weeks (HOMA-IR 1.7±1.4 vs 1.3±0.7, $p<0.05$), and was now similar to normal weight subjects ($p=0.654$). Leptin concentrations fell

significantly (baseline 61±35ng/ml to 26±17ng/ml, p<0.001), and to values not significantly different to normal controls despite BMI remaining in the obese range.

There was no overall change in adiponectin concentrations (baseline 41±50μg/ml, week 8 38±67 μg/ml, p=0.191), although this may reflect a combination of improved insulin sensitivity with reduction in body fat content. The change in leptin correlated with change in total body fat mass, whereas the alteration in adiponectin concentration was associated with fall in visceral fat volume (figure 3.7). However, there was no statistically significant association between change in the measured adipokines and any cardiac structural or functional parameter.

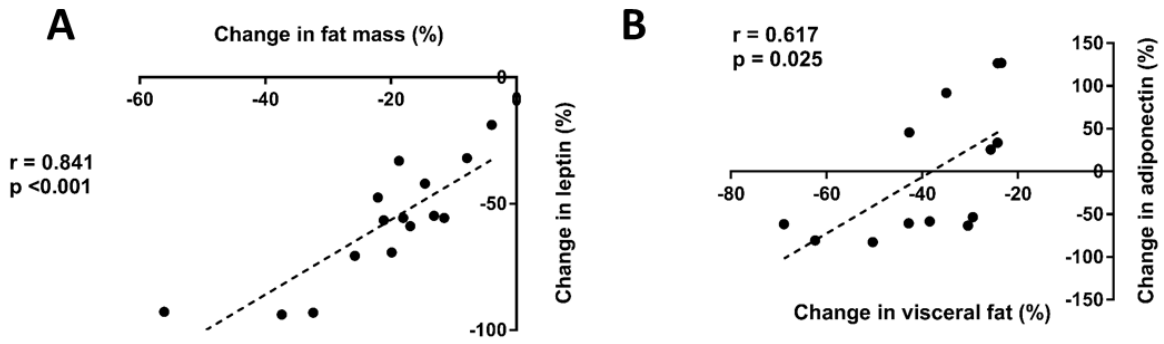


Figure 3.7: The change in leptin concentrations with weight loss (A) correlates with change in total fat mass, whereas the change in adiponectin concentration (B) correlates with change in abdominal visceral fat area

Final blood pressure values were significantly lower than baseline (systolic 124±12mmHg, p=0.035; diastolic 80±10mmHg, p=0.068), and again similar to normal values (control group systolic blood pressure 121±15 mmHg, p=0.192; diastolic 77±11mmHg, p=0.848). Resting heart rate continued to fall (to 59±7bpm, p=0.010 compared to baseline).

β-hydroxybutyrate remained significantly elevated (0.46±0.23 mmol/l compared to 0.25±0.10 mmol/l, p=0.014) and FFAs continued to rise (week 8 277±136 μmol/l vs

week 1 168±61μmol/l, p=0.018, figure 3.6B). However, having initially increased from baseline at 1 week, by 8 weeks myocardial triglyceride content had now fallen, and were now significantly lower than recorded baseline (p=0.050, fig 3.8A).

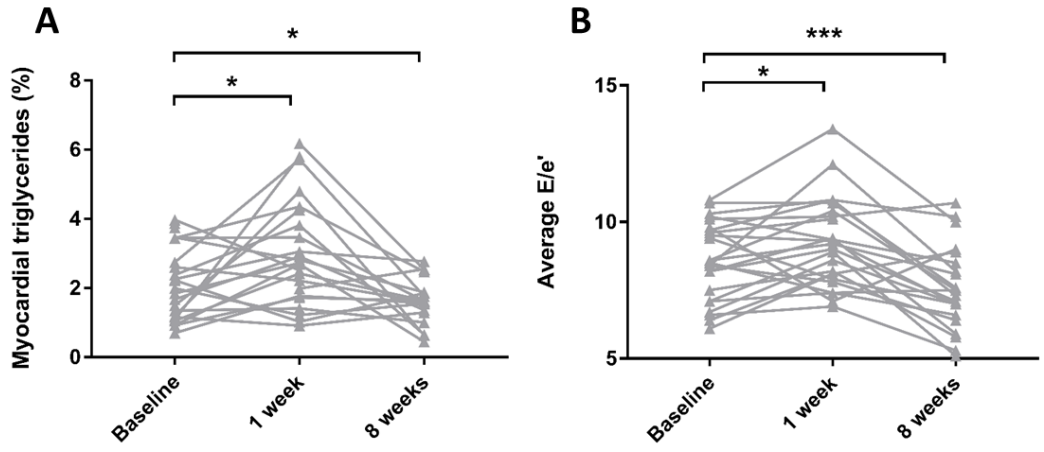


*Figure 3.8: Myocardial triglycerides and LV diastolic function at 8 weeks compared to baseline and 1 week. * indicates p<0.05, *** p<0.001.*

By 8 weeks, LV geometry was improved; EDV and mass were significantly lower than baseline, and not statistically different from normal weight controls (p=0.403 for EDV and p=0.222 for mass). Systolic function had returned to normal (LV EF 64±5% compared to 66±5% baseline, p=0.638). Not only had diastolic function improved from the 1 week time-point (E/e' 8 weeks 7.5±1.5 vs 1 week 9.4±1.7, p<0.001), but there was a significant improvement from the mildly impaired baseline (E/e' 8 weeks 7.5±1.5 vs baseline 8.6±1.4, p=0.003, fig 3.8B), with values similar to normal controls (p=0.877).

Whilst initially impaired, myocardial energetics had now improved over the course of the VLCD (PCr/ATP by 14%, p=0.021) and were now similar to normal values (study group 2.2±0.2 compared to controls 2.2±0.2, p=0.540).

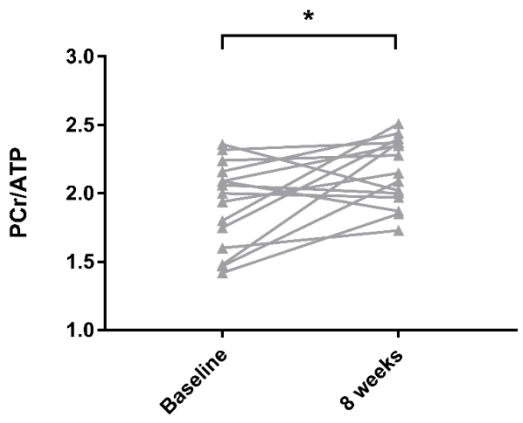


*Figure 3.9: PCr/ATP at baseline and after 8 weeks of a Very Low Calorie Diet. * p<0.05.*

By 8 weeks, aortic distensibility returned to baseline levels in both ascending and descending portions (ascending p=0.262, descending p=0.65), but were still impaired when compared to controls (5.0±2.9mmHg^{-1} compared to normal 7.3±2.4 mmHg^{-1}, p=0.050).

3.4 Impact of diet in impaired baseline diastolic function

In order to assess both degree of improvement at 8 weeks, and the deterioration at the early time-point in individuals in whom diastolic function is already impaired, the cohort was divided around the mean baseline E/e' ratio (n=13<8.5; n=11>8.5). The two cohorts were well-matched for baseline characteristics, other than diastolic function, and higher levels of visceral fat in the diastolic impairment group (196±89cm^2 compared to 128±58 cm^2, p=0.045).

Although the changes seen in both liver fat and myocardial triglycerides at one week were similar between the two groups (liver fat -2.5±1.3% in normal diastolic function, -2.3±2.0% in impaired; MTGC +1.3±1.8% in normal, +1.3±1.5% in impaired, all p>0.05), the cardiovascular functional deterioration at one week was almost entirely limited to the normal diastolic function group, with both systolic function (absolute LV

EF fell by 4±6%, p=0.089; peak radial strain by 6±7%, p=0.027) and diastolic function (average E/e' rose from 7.5±0.8 to 8.6±1.4, p=0.035) deteriorating to a greater extent than in the impaired group (no statistically significant changes in the above parameters).

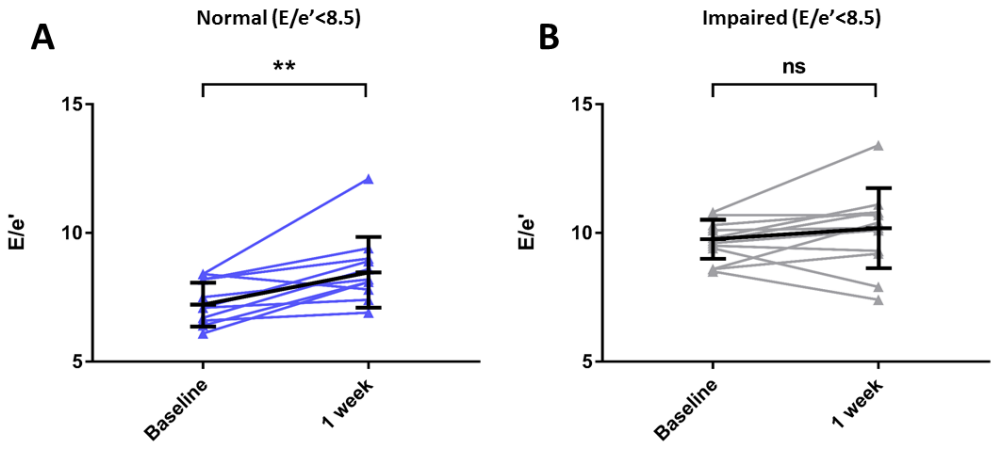


*Figure 3.10: The early deterioration in diastolic function is limited to those with normal diastolic function (A). ** p<0.01.*

However, whereas there were minimal acute functional changes in the diastolic impairment group, the functional improvement seen at 8 weeks were more pronounced in this group (average E/e' fell from 9.6±0.8 to 8.4±1.4, p=0.013) despite similar changes in overall body fat and visceral fat distribution.

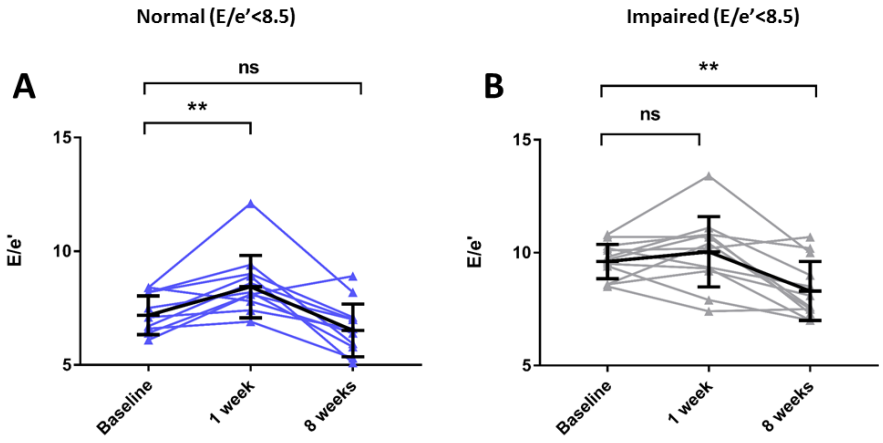


*Figure 3.11: The improvement from baseline in diastolic function is more pronounced in the group with impaired baseline function (B). ** indicates p<0.05.*

4. Discussion

Having lost favour in the 1970s, there has recently been a renaissance of Very Low Calorie Diets as a means to combat excess body weight and reverse the metabolic consequences of obesity, both by physicians and by the general public in a semi-regulated fashion. The recent DiRECT trial, showing effectiveness in diabetes reversal[193] should undoubtedly increase their utilisation further. Despite these established metabolic improvements, by elevating free fatty acid (FFA) levels, VLCDs have the potential to cause cardiovascular decline through manipulating myocardial substrate utilisation. These results demonstrate a transient, early and significant deterioration in cardiovascular function, with increased MTCG after one week of comparatively modest caloric restriction (6-800kcal per day). The cardiovascular changes are in contrast to the more commonly measured parameters of body fat, insulin resistance, and hepatic steatosis, which improve from the onset of the diet. These findings have particular significance for those with co-existing cardiac pathology, those who are undertaking these diets as part of the pre-operative preparation immediately preceding bariatric surgery, and those following these programmes independently without supervision. Despite this early deterioration and final BMI remaining in the obese category, by 8 weeks diastolic function had improved to normal values, suggesting that, if adhered to for a long enough period of time, VLCDs may be a therapeutic option for diastolic dysfunction in diabetes and obesity.

4.1 Early cardiovascular deterioration in Very Low Calorie Diet

In this study, it is reiterated that successful weight loss over the course of 2 months in obesity results in substantial cardiovascular benefits with reduced LV mass, improved

myocardial energetics, reduced myocardial triglyceride content, improved diastolic function, and increased aortic elasticity. However, initially there is an increase in myocardial steatosis (by 43%) that is accompanied by reduced LVEF, and diastolic dysfunction (with increased E/e'). Profound caloric restriction leads to rapid mobilisation of lipid stores, in order to maintain blood glucose levels following exhaustion of glycogen stores through hepatic gluconeogenesis using the glycerol group of stored triacylglycerols. This results in a rapid rise in circulating FFA levels, and as myocardial uptake of FFA is proportional to plasma concentration (via a combination of passive diffusion and regulated active transport[202]), the relative steatosis is likely to reflect a combination of increased rate of FFA uptake into the myocardium, as well as a degree of impairment of FFA oxidation in the context of insulin resistance.

This increase in lipid content is likely to contribute to the functional deterioration, and MTGC has been previously shown to contribute to cardiac dysfunction in human obesity[203]. Consistent with this, the increase in MTGC is related to a deterioration in systolic and diastolic function at the early time-point. In addition, the fall in LV end-diastolic volume, in the context of elevated diastolic markers and absence of volume depletion, is likely to represent stiffening of the left ventricle. Reassuringly, BNP did not elevate at this time-point; however, this is consistent with the fact that LVEF did not drop below the normal range, and furthermore, is difficult to interpret given the negative association between obesity and BNP[204].

4.1.1 Potential mechanisms for early cardiovascular deterioration

There are a number of mechanisms which could contribute to the early deterioration in function, including altered substrate selection, altered adipokine environment and a change in autonomic balance.

One of the main candidates is increased levels of MTGC. In addition to being a less energy efficient substrate than glucose[84], the oversupply of FFAs beyond the oxidative capacity of the heart leads to diversion of triglyceride down non-oxidative pathways and accumulation of lipotoxic intermediate metabolites such as ceramide and diacyl glycerol. These not only further impair FFA oxidation but also worsen intracellular insulin sensitivity, alter calcium handling[205] and favour accumulation of reactive oxygen species (ROS)[74], all of which impair cardiac function[72].

The data in this experiment demonstrated a relationship between a greater degree of systolic impairment at 1 week, and lower levels of circulating ketones, consistent with the theory that the myocardium will preferentially utilise ketones where available, associated with greater mitochondrial efficiency. However, there is also recent evidence to suggest that myocardial uptake of ketones is both increased in insulin resistance, and that this is associated with LV dysfunction[206], indicating that this is a complex relationship and worthy of further investigation.

The acute changes in cardiac function are more pronounced in a metabolically healthier cohort, with lower levels of abdominal visceral fat, better insulin sensitivity, and normal baseline diastolic function, despite similar changes in fat mass and distribution, hepatic lipid and circulating FFAs. This may reflect the acute effects of imposing fatty acid

utilisation on an insulin sensitive myocardium, rather than adaptation to the gradual pathophysiological process of deteriorating substrate flexibility over months and years.

The adipokine profile in an individual may provide a plausible link between adipose tissue and myocardial metabolism. The metabolic effects of leptin and adiponectin are mediated by various pathways including the key regulator of substrate selection, AMPK. Insulin resistance[207] and inflammation[208] suppress adiponectin release, so it is possible that it is a marker of a cluster of factors including insulin resistance, dysfunctional adipose tissue distribution, ectopic lipid, and baseline diastolic dysfunction – none of these factors in isolation predict the functional change at one week, so a direct role for adiponectin in mediating susceptibility to substrate changes is potentially implicated.

In a similar fashion to myocardial function, aortic elastic function becomes impaired at this early time-point. As there is evidence both that raised FFAs increase ROS in arterial endothelial cells and impair endothelial function[209], and that artificial elevation of FFAs results in a similar pattern of aortic stiffness[196], it is likely that this is involved in the vascular stiffening seen at one week. In addition, intralipid-heparin infusion-induced elevated free fatty acid levels are known to decrease endothelium-dependent vasodilation, nitric oxide production[210-212], decrease endothelial nitric oxide synthase activity and impair endothelium-dependent vasodilation, supporting the concept that this may be the mechanism acutely[15, 16].

Overall, despite early, profound metabolic improvements in glucose, cholesterol, autonomic function in terms of blood pressure and heart rate, and insulin resistance, there is an initial deterioration in LV systolic and diastolic function associated with early increases in MTGC and aortic stiffness when following a VLCD. Whilst the majority of

subjects are likely to tolerate this magnitude of change, to what extent individuals with established heart failure would be affected by these changes is unknown.

4.2 Cardiovascular benefits after 8 weeks of VLCD

By 8 weeks, with ongoing and significant reduction in fat mass and improved whole-body insulin sensitivity, myocardial triglyceride levels fall below baseline levels. This suggests that ongoing dietary weight loss enables MTGC to be utilised by the heart, and hence intracellular levels are able to normalise despite ongoing circulating FFA excess.

The normalisation of MTGC is associated with improvements in both systolic function, which returns to baseline levels, and diastolic function, where E/e' is seen to improve at this stage to a level which is both better than baseline, and similar to normal weight controls. This is most marked in individuals with baseline elevated visceral fat and associated IR, and pre-existing diastolic dysfunction.

4.2.1 Mechanisms of improved cardiovascular function with weight loss

Interestingly, the cohort remains obese in terms of fat mass and BMI after the VLCD intervention, but myocardial energetics and MTGC have returned to normal levels. This, together with the deterioration at one week despite weight loss, strongly suggests that the energetic and functional impairment seen in obesity is driven by altered cardiac metabolism. The fall in MTGC despite persistently increased circulating FFAs also suggests that the myocardium must be increasing oxidative capacity for FFAs by 8 weeks. As peroxisome proliferator-activated receptor alpha (PPAR-α) is the primary regulator of FA oxidation in the heart[60], and has paradoxically been shown to have a blunted response to FAs in obesity[76], it is possible that increased PPAR-α activity in response to

improved insulin resistance and reduced fat mass, is responsible for the decrease in MTGC seen here.

It is known that actions of adipokines such as leptin and adiponectin is mediated by modification of intracellular pathways such as AMPK and increased expression of PPAR-α, and it may be the change in secretion and/or sensitivity to these hormones which mediates alterations in substrate metabolism. However, while a significant fall in leptin is seen with total fat mass reduction in this data, there is no demonstrable link between the magnitude of this change, and improvement in metabolic or functional capacity. Additionally, overall there was no change in adiponectin concentrations despite significant reduction in body fat mass and improvement in insulin sensitivity. This may reflect the complex relationship balancing relative volumes of dysfunctional, insulin resistant adipose tissue which is resistant to adiponectin. The association between greater reduction in visceral fat and fall in adiponectin levels is consistent with the theory that reduction in dysfunctional insulin resistant adipose tissue, improves adiponectin sensitivity and hence is conversely associated with a fall in circulating levels. However, again, there was no direct correlation between change in adiponectin and any cardiovascular functional parameter, suggesting that further investigation of this complex relationship is warranted.

It is also interesting to note that despite the echocardiographic evidence of improved diastolic function, BNP levels in fact rise by a small but significant proportion, in keeping with the likely effect of reduction in fat mass on BNP metabolism[204].

Aortic elastic function also follows a time dependent course, returning to baseline function at 8 weeks, but remaining impaired when compared to controls. As it is known

that aortic distensibility is improved after one year of weight loss[58], this suggest that a longer time course is needed to fully reverse aortic stiffness, potentially to a point when adipose tissue stores are reduced such that fatty acid levels fall below pre-diet levels.

In summary, after 8 weeks of VLCD, there are continued metabolic improvements in fasting glucose, cholesterol, blood pressure and insulin resistance, accompanied by ongoing reductions in total, abdominal visceral, epicardial and liver fat. Despite initial increase in myocardial fat and deterioration in cardiovascular function, by the end of the VLCD myocardial energy metabolism and diastolic function return to normal.

4.3 Study limitations

While the results measured in these experiments are sound, there are several areas of limitation, or rather potential over-simplification, which should be considered in drawing any definite mechanistic conclusions.

While an attempt to ensure dietary adherence was made by assessing circulating ketone levels as well as monitoring frequency of supplement delivery, clearly variation in individuals' commitment and caloric intake was still possible in a community-based study. Plasma osmolality was not measured, and so the impact of caloric restriction on this was not assessed – however, expected shifts would be very small as this is so tightly regulated, and so the study may not have been sufficiently powered for this.

HOMA-IR is clearly an estimate of whole-body insulin sensitivity, and could be argued to be inevitably affected by altering dietary intake. Additionally, we now appreciate that there are significant differences in tissue-specific insulin sensitivity, meaning that

HOMA-IR should not necessarily be used to infer information about individual organ behaviour.

In a similar fashion, while circulating leptin and adiponectin concentrations are interesting and associated with a significant evidence base in themselves, we now know that both expression and sensitivity vary in different tissue types, and hence caution should again be used in over-interpreting any changes. While *in vivo* tissue-specific assays of hormone sensitivity rely on biopsy, this may be more straightforward in, for example, skeletal muscle or subcutaneous adipose tissue rather than myocardium, and is a worthwhile consideration when planning future work.

5. Conclusions

Although Very Low Calorie Diets produce profound and rapid improvements in insulin resistance and hepatic steatosis, they are associated with an early but significant deterioration in cardiac function, metabolism and aortic elasticity. This impairment is transient, and with ongoing reduction in body fat over the course of a full 8 week programme, improvements in cardiac function become evident. As VLCDs are aimed at reversing diabetes and obesity, and used routinely pre-operatively for bariatric surgery, they are targeted to populations who may well have coexisting cardiac disease or are following these programmes independently in the community; this data suggests that early medical supervision may be necessary for VLCDs in those with cardiovascular disease. Additionally, further assessment of individuals prior to bariatric surgery may be required in view of the early deleterious changes. However, these dietary interventions remain an exciting potential therapeutic strategy for the treatment of obesity-related heart disease.

Additionally, this evidence underlines the importance of substrate selection in mediating the relationship between body fat and cardiovascular function, and the potential for targeting metabolic and/or autonomic pathways in the treatment of diabetic and obesity-related cardiomyopathy.

Chapter Four

The Impact of Obesity on Myocardial Creatine Kinase Kinetics

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1. Background

Obesity is associated with myocardial functional impairment, ranging from subclinical diastolic dysfunction to overt systolic impairment. This is due to a combination of numerous factors, which also include relative inflexibility in substrate utilisation, with increased fatty acid uptake and oxidation in comparison to glucose, which has associated effects on cardiovascular function as demonstrated in the previous chapter. In addition to the direct lipotoxic damage caused by this imbalance in myocardial metabolism, its overall efficiency is reduced[84], as energy yielded by fatty acid oxidation comes at a greater oxygen cost. Over time, this combination of increased work in the context of inefficiency leads to depleted concentrations of creatine within the myocardium. The relative sizes of substrate pools can be measured non-invasively with phosphorus magnetic resonance spectroscopy, represented by the ratio of phosphocreatine to ATP (PCr/ATP). This value has been demonstrated to be depleted in obesity[8].

However, measuring a single time point of relative energy stores tells us little about the actual rates of ATP delivery at the level of the myofibril where cardiac work is undertaken. It has been shown that even in the presence of depleted PCr/ATP, we can glean additional, more sensitive information by measuring the rate of phosphotransfer

via the creatine kinase pathway. This pathway provides a spatial buffer to the delivery of ATP, the rate of which is determined by the catalytic activity of creatine kinase, both mitochondrial and cytosolic. Its activity has been demonstrated to be specifically reduced in individuals with heart failure compared to those with asymptomatic hypertrophy[93], and hence may be implicated in the decompensation towards failure.

Myocardial creatine kinase activity has never been measured in human obesity. However, there are some interesting data suggesting that, instead of activity being reduced as is seen a range of other cardiomyopathies[96], obesity and insulin resistance may actually be associated with a compensatory increase in creatine kinase activity[213, 214].

I sought to establish: 1) whether this was the case in the human heart even prior to the development of systolic impairment; 2) whether the response of creatine kinase to dobutamine stress unmasks more subtle abnormalities; 3) whether any cardiac changes seen in obesity are related to the underlying energetic changes; 4) whether the exercise limitation seen in obesity is related to any measured resting or dynamic energetic abnormalities, and 5) to what extent any measured changes are reversed by weight loss.

2. Methods

For detailed methods, see Chapter Two. The study was approved by the local ethics board (NRES reference 14/SC/004). 72 volunteers were recruited from the Oxfordshire community, 27 of whom were normal weight (body mass index (BMI) 18.0-25.0 kg/m^2) and 45 obese (BMI >30m/kg^2). All signed written consent for the study investigations.

2.1 Inclusion criteria

Participants were excluded if they had any history of heart failure, coronary artery disease or severe valvular disease. Additionally, uncontrolled hypertension, atrial fibrillation or diabetes mellitus were exclusion criteria, as was any contraindication to MR scanning.

2.2 Anthropomorphic and biochemical assessment

Height, weight and body composition were measured using digital scales with bio-impedance analysis. A standard 12-lead electrocardiogram was recorded, and non-invasive blood pressure measured. Fasting venous blood was drawn to measure circulating biomarkers.

2.3 Magnetic resonance imaging

All MRI was performed at 3T (Siemens Tim Trio, Siemens Erlangen). Abdominal visceral fat, hepatic fat fraction, cardiac structure and systolic function, and myocardial triglyceride content using ^{1}H spectroscopy were acquired as described in Chapter Two.

2.3.1 Phosphorus spectroscopy

^{31}Phosphorus spectroscopy was performed on the same 3T system described above. Creatine kinase k_f was measured using a modified TRiST sequence[183].

The relative ratio of PCr to γ-ATP peaks in the fully relaxed spectrum is used to generate a PCr/ATP ratio, and this value multiplied by literature values for [ATP] (5.7 μmol/g in normal systolic function, 5.2 μmol/g in heart failure)[93] to calculate [PCr]. Overall ATP delivery, or CK flux, was then calculated by [PCr]* k_f.

In order to measure the effect of catecholamine stress on CK k_f, the STreST protocol was acquired under low-dose dobutamine stress according to the protocol described in Chapter Two.

^{31}P-MRS data was analysed using AMARES analysis software within Matlab.

2.4 Echocardiography

Echocardiography was performed on a Philips Epiq system, to determine diastolic function; pulse wave velocity was measured at mitral valve inflow and tissue Doppler at lateral and medial mitral valve annulus.

2.5 Cardiopulmonary exercise testing

Volunteers underwent cardiopulmonary exercise testing at each visit, with measurement of maximal capacity on bicycle ergometer, and submaximal capacity on six minute walk test assessment.

2.6 Weight loss intervention

The obese volunteers underwent dietary weight loss advice with telephone/email support from the study team. 20 participants adhered to a low glycaemic index, calorie controlled diet over a period of 376 days (IQR 315-466 days). The remaining 25 underwent an initial Very Low Calorie Diet (as described in Chapter Three), for 8

weeks, before following a calorie controlled maintenance diet as per the other group. Volunteers were encouraged to maintain current activity levels during this timeframe.

2.7 Statistical analysis

Statistical analysis was performed using commercial software (SPSS 24, Chicago). All data is presented as mean ± standard deviation unless stated. Equality of variance was confirmed using Levene's test, before determination of statistical significance, either by Chi-squared tests in the case of categorical data, or student's t tests for continuous data (paired or independent where relevant). Non-parametric data was analysed with the Mann Whitney U test, or Kruskal Wallis test. Pearson's correlation and linear regression were used. Values of $p<0.05$ were considered as statistically significant.

3. Results

As expected, obese volunteers had significantly higher BMI (35±5 kg/m^2 vs 23±2 kg/m^2; p<0.001) and total body fat mass (44±11 kg vs 16±7 kg; p<0.001) compared to normal weight volunteers. The two groups were however well matched for age and sex (see table 4.1). Blood pressure were significantly higher in the obese group (systolic 136±19 mmHg vs 123±19 mmHg in controls, p=0.008; diastolic 85±12 vs 74±13, p=0.001), as was resting heart rate (67±13 vs 57±8, p=0.001**).** Total cholesterol, triglycerides, glucose and insulin were also all significantly higher in the obese group (table 5), as was leptin. 27 of the 45 (60%) volunteers would be defined as insulin resistant (HOMA-IR>2.0[215]), and 18 (40%) fulfilled the criteria for a diagnosis of non-alcoholic fatty liver disease (>5% liver fat). Abdominal visceral fat was significantly elevated compared to normal controls (156±81cm^2 compared to 49±42cm^2, p<0.001). Interestingly, despite measured differences in diastolic function, there was no significant difference between baseline BNP readings (normal 7±5 pg/ml compared to obese group 5±5 pg/ml, p=0.183).

Table 4.1: Baseline anthropomorphic and metabolic findings

	Normal weight n=27	Obese n=45	p
Age (y)	43±17	48±15	0.165
Female (%)	14 (52)	32 (74)	0.422
BMI (kg/m^2)	23.1±2.4	35.4±4.7	**<0.001**
Weight (kg)	66±10	101±17	**<0.001**
Body fat mass (kg)	16±7	44±11	**<0.001**
Systolic blood pressure (mmHg)	123±19	136±19	**0.008**
Diastolic blood pressure (mmHg)	74±13	85±12	**0.001**
Metabolic status:			
Visceral fat area (cm^2)	47±42	156±81	**<0.001**
Liver fat (%)	0.9±0.6	6.9±6.3	**<0.001**
Myocardial triglyceride (%)	1.0±0.7	2.2±1.7	**0.023**
Total cholesterol (mmol/l)	4.6±1.0	5.4±1.1	**0.026**
Triglycerides (mmol/l)	1.0±0.5	1.7±0.9	**0.004**
Fasting glucose (mmol/l)	4.8±0.3	5.4±0.7	**0.002**
Fasting insulin (mmol/l)	50±25	106±89	**0.001**
HOMA-IR	1.4±0.7	3.9±4.0	**0.023**
BNP (pg/ml)	7±5	5±4	0.183
Leptin (ng/ml)	23±12	57±34	**<0.001**

3.1 The impact of obesity on cardiac structure and function

In line with previous reports, obesity was associated with concentric LV remodelling with elevated LV mass (114±27g compared to 99±18g; p=0.019, table 4.2), increased mass-to-volume ratio (0.73±0.13 compared to 0.68±0.07; p=0.036) and a trend towards increased end-diastolic volume (158±33ml compared to 147±24ml; p=0.140). Systolic

function was relatively hyperdynamic in the obese group, with ejection fraction 66±4% compared to 62±5% in the normal weight group (p=0.003), and systolic radial strain similarly elevated (49±11% compared to 41±7%, p=0.001). There was relative diastolic dysfunction in the obese group (E/e' 9.1±2.0 vs 7.6±1.4, p=0.044).

3.2 Obesity and exercise capacity

64 participants underwent cardiopulmonary exercise testing, with all achieving adequate tests, and no difference between the groups in terms of effort (RER normal weight 1.14±0.09 vs obese 1.12±0.07, p=0.125; percentage of maximum heart rate normal weight 91±7% vs obese 89±10%, p=0.351, table 4.2).

The obese group were more limited in terms of maximal performance, with lower peak $\dot{V}O_2$ at 20±5 ml/kg/min (compared to normal weight 33±9 ml/kg/min, p<0.001), and lower percentage of predicted $\dot{V}O_2$ achieved (87±19% compared to control 116±27%, p<0.001). Both these parameters are calculated using body weight, but even using less body weight dependent variables such as total exercise time and heart rate reserve, indicating relative chronotropic incompetence, the obese group were relatively impaired (exercise time 9±2 mins vs control 11±3 mins, p=0.010; HRR 0.7±0.2 vs control 0.8±0.1, p=0.040). There was however no difference between six minute walk test distance, ventilatory efficiency slope ($VE/\dot{V}CO_2$) or $\dot{V}O_2$ indexed to fat-free mass between the two groups.

Table 4.2: Baseline cardiac parameters and exercise capacity

	Normal weight n=27	Obese n=45	p
Left ventricular parameters:			
End-diastolic volume (ml)	147±24	158±33	0.140
End-systolic volume (ml)	55±11	54±14	0.660
Cardiac output (l/min)	5.1±0.8	6.6±1.8	**<0.001**
Mass (g)	99±18	113±27	**0.019**
Ejection fraction (%)	62±5	66±4	**0.003**
Mass-volume ratio	0.68±0.07	0.73±0.13	**0.036**
E/e'	7.6±1.4	9.1±2.0	**0.044**
Exercise capacity:			
Peak $\dot{V}O_2$ (ml/kg/min)	33±9	20±5	**<0.001**
$\dot{V}O_2$ indexed to fat-free mass (ml/kg/min)	41±10	36±7	0.123
Percentage of predicted $\dot{V}O_2$ achieved (%)	116±27	87±19	**<0.001**
Exercise time (min)	11±3	9±2	**0.010**
RER	1.14±0.09	1.12±0.07	0.125
VE/$\dot{V}CO_2$	28±4	26±4	0.226
Heart rate reserve	0.8±0.1	0.7±0.2	**0.040**
Six minute walk test distance (m)	599±32	579±68	0.173

3.3 Effect of obesity on myocardial energetics

Obesity was associated with reduced PCr/ATP (1.93±0.31 compared to 2.19±0.22; p<0.001). Analysing both groups, PCr/ATP was inversely related to age (r -0.414, p=0.001), visceral fat (r -0.304, p=0.021, figure 4.1A), myocardial triglyceride content (r -0.423, p=0.004), and resting ejection fraction (r -0.252, p=0.046).

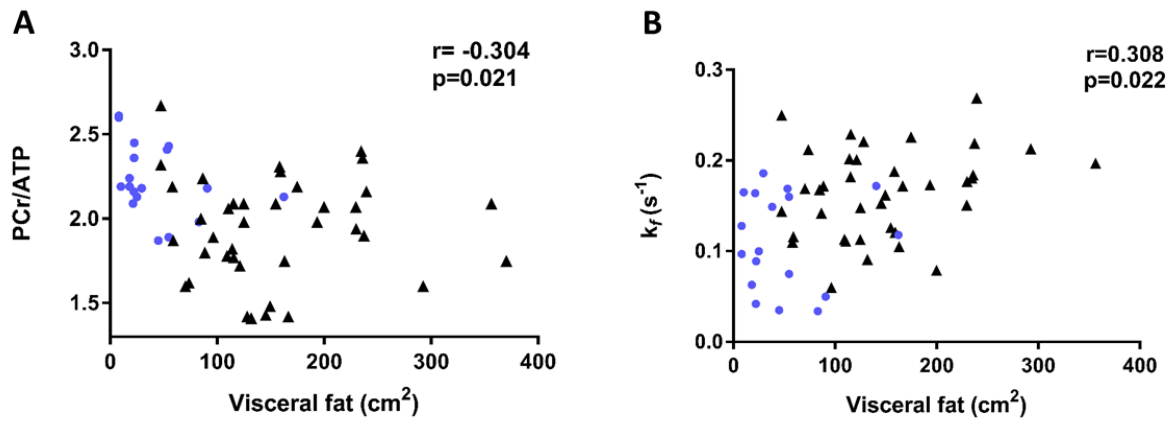


Figure 4.1: The contrasting effect of increasing visceral fat on PCr/ATP (A) and creatine kinase kinetics (B) (normal controls in blue, obese in black)

If these parameters are entered into a multiple regression, only MTGC remains significant (β -0.451, p=0.003; table 4.3).

Table 4.3: Determinants of myocardial PCr/ATP

	Simple regression		Multiple regression	
	R	p	β	p
MTGC	-0.457	0.002	-0.451	0.003
Age	-0.414	0.001	-1.688	0.100
Ejection fraction	-0.252	0.046	-0.757	0.454
Visceral fat	-0.304	0.021	-0.93	0.926

In contrast, the forward rate constant of creatine kinase (CK k_f) was increased in the obese group when compared to normal weight (0.163±0.049s^{-1} versus 0.119±0.057, p=0.001). Similarly, CK k_f was positively correlated with BMI (r 0.375, p=0.003), fat mass (r 0.393, p=0.005), visceral fat (r 0.316, p=0.020, figure 4.1B) and leptin concentration (r 0.355, p=0.012), but not with age, MTGC or any resting functional parameter.

The increase in CK k_f compensated for the depleted PCr/ATP stores when considering ATP delivery (CK flux) with overall values showing a trend towards increased levels in obesity than normal weight individuals (1.8±0.6 μmol/g/sec compared to 1.4±0.7 μmol/g/sec; p=0.063).

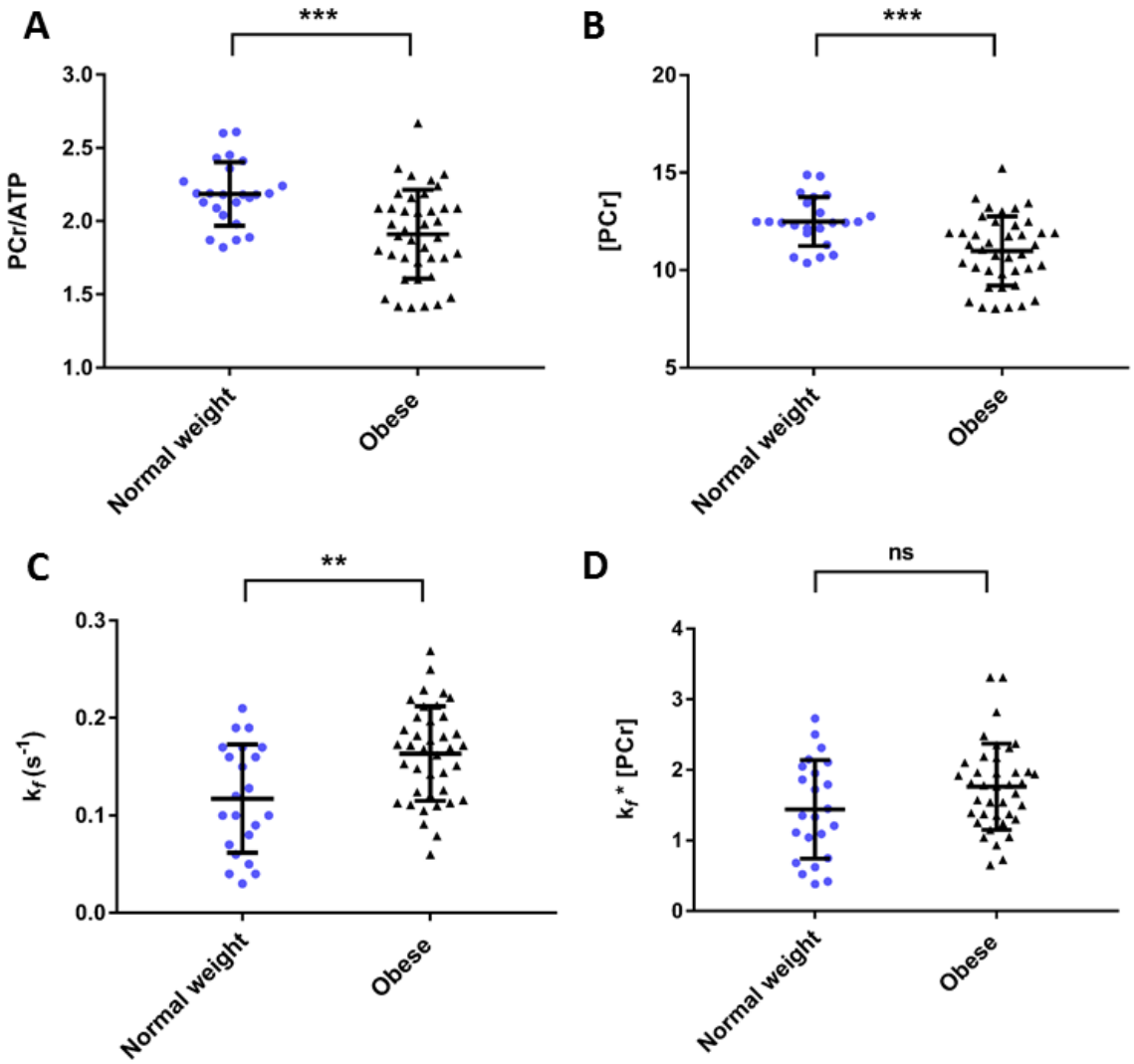


*Figure 4.2: Obesity is associated with lower PCr/ATP values (A), PCr concentration (B), increased forward rate constant of the creatine kinase reaction (C) and a trend towards increased CK flux (D). ** indicates p<0.01; *** indicates p<0.001.*

I then considered the CK kinetics in the context of the amount of cardiac work being done, given that the obese group had relatively higher left ventricular stroke work (LV stroke volume*systolic blood pressure; 10.2±2.4 l*mmHg compared to 8.1±2.0 l*mmHg,

p=0.001) and rate-pressure product (HR*systolic blood pressure) at rest. I indexed CK k_f both to left ventricular stroke work and RPP, and demonstrated no significant difference between control and obese groups (k_f /stroke work p=0.123, k_f /RPP p=0.734). Additionally, there was a correlation between resting CK k_f and left ventricular stroke work (r 0.286, p=0.042), suggesting that the CK rate constant increases with increasing energetic demand within the tissue. The impact of increasing cardiac work on creatine kinase kinetics was further examined using dobutamine.

3.4 Effect of catecholamine stress

38 participants (21 normal weight and 17 obese) underwent dobutamine stress. This was well-tolerated, with no significant adverse effects and only 3 volunteers not completing the protocol due to either nausea (n=2) or discomfort (n=1). Mean heart rate was 65±6% of maximum, with a 152% increase in RPP being observed. This was slightly lower in the obese cohort, due to higher baseline blood pressure, but did not reach statistical significance (table 4.4).

Dobutamine led to significant increases in ejection fraction in both obese (by 20±11%, p<0.001) and control groups (by 29±10%, p<0.001). However, the increase in normal weight controls was relatively greater (p=0.023) likely reflecting the increased baseline ejection fraction in obesity.

Table 4.4: Haemodynamic response to stress

	Normal weight n=20	Obese n=15	p
Total duration dobutamine (mins)	24±3	25±2	0.676
Resting heart rate (bpm)	57±7	64±9	**0.012**
Stress heart rate (bpm)	113±8	114±10	0.858
Average heart rate (% maximum)	64±4	67±6	0.100
Resting systolic blood pressure (mmHg)	121±17	133±15	**0.029**
Maximum systolic blood pressure (mmHg)	151±14	168±19	**0.005**
Resting RPP (mmHg*bpm)	6899±1293	8591±1595	**0.001**
Stress RPP (mmHg*bpm)	17264±1793	19071±2085	**0.010**
Relative increase in RPP (%)	167±60	132±38	0.079
Relative increase in LV stroke work (%)	24±35	21±27	0.818

3.4.1 Effect of catecholamine stress on myocardial energetics

In contrast to previous reports, there was no recorded change in PCr/ATP with dobutamine stress in either group (normal weight 2.2±0.2 at rest to 2.2±0.4 at stress (p=0.463); obese 1.9±0.3 at rest to 1.8±0.5 at stress, p=0.666). However, CK k_f increased with dobutamine stress in the normal weight group, by 72±77% (p=0.002), and overall CK flux also increased, by 69±83% (p=0.007). In contrast, in the obese group despite similar increases in left ventricular stroke work, no significant increase was observed in either CK k_f (rest 0.14±0.06s^{-1} vs stress 0.13±0.06s^{-1}, p=0.117) or the product of $k_f$and [PCr] (rest 1.5±0.7 μmol/g/sec vs stress 1.4±1.0 μmol/g/sec, p=0.608; figure 4.3).

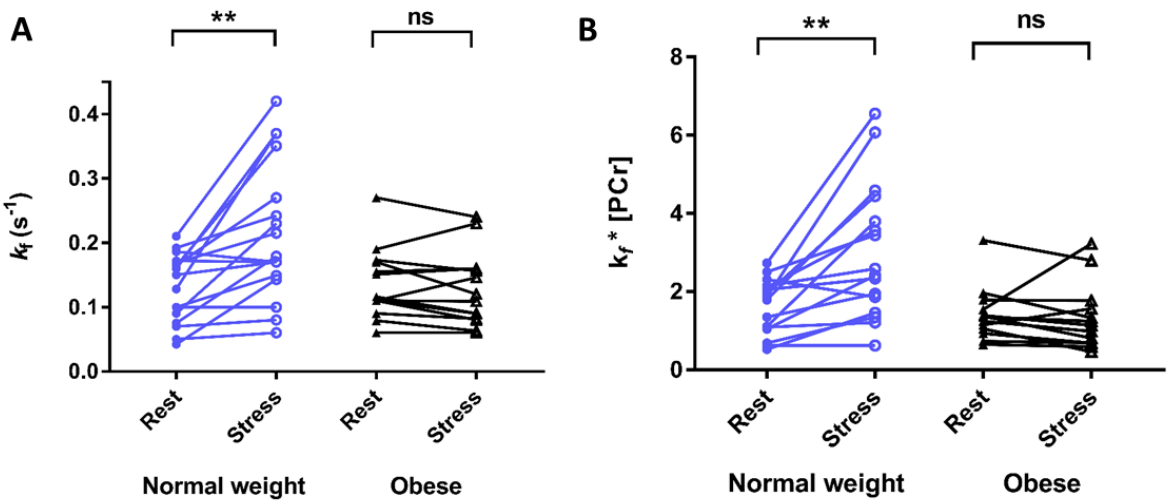


*Figure 4.3: The effects of catecholamine stress on CK k_f (L) and ATP delivery (R) in normal weight and obese individuals. ** indicates p<0.01, *** p<0.001.*

3.5 Relationship between myocardial energetics and exercise capacity

Considering both controls and obese cohorts together, the change in CK k_f in response to dobutamine stress correlates with exercise capacity, either measured by peak $\dot{V}O_2$ (r 0.659, p<0.001, Figure 4.4A), or heart rate reserve (the ratio of the observed change in heart rate compared to the potential increase seen – essentially a marker of chronotropic competence; r 0.555, p=0.006, Figure 4.4B). Given the non-significant change in CK k_f with stress in obesity, this is primarily driven by the normal controls (peak $\dot{V}O_2$ r 0.622, p=0.031; HRR r 0.484, p=0.131), with no significant relationship observed in obesity (peak $\dot{V}O_2$ r -0.049, p=0.867; HRR r 0.168, p=0.375).

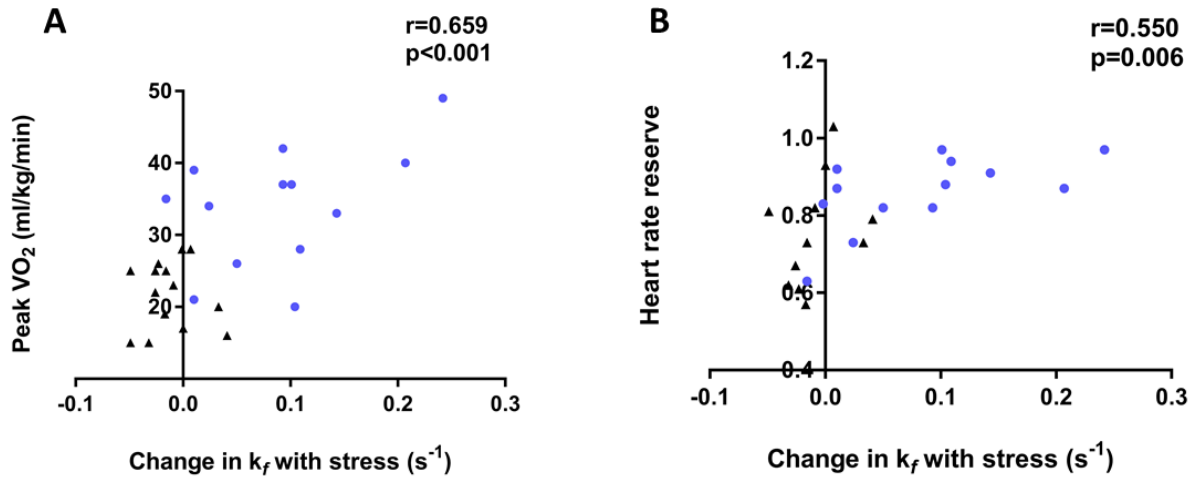


Figure 4.4: The change in creatine kinase k_f with dobutamine stress correlates with both peak $\dot{V}O_2$ (A) and heart rate reserve (B). Normal controls in blue, obese in black.

3.6 Effect of weight loss

Of the obese cohort (n=45) who underwent the weight loss intervention, 9 (20%) declined follow-up, and, of the remaining 36, 27 (75%) lost weight (as defined by any reduction in body weight) over the intervention, with a mean weight loss of 11±5% and fat mass loss of 23±14% (table 4.5). The remaining 9 participants' weight remained unchanged (+4±5%, p=0.113).

Considering those individuals who succeeded in losing weight, metabolic blood tests improved, with cholesterol falling by 13±16% (p=0.001), fasting glucose by 9±11% (p=0.001) and HOMA IR-by 41±33% (p=0.003). Systolic blood pressure fell from 134±21 mmHg to 126±12 mmHg albeit not reaching statistical significance with this sample size (p=0.085).

Table 4.5: Anthropomorphic and metabolic consequences of successful weight loss

	Normal weight controls n=24	Obese at baseline n=27	Obese post weight loss n=27
BMI (kg/m^2)	23.6±2.4	36±6.0	32±5***
Body weight (kg)	66±10	105±19	94±18***
Body fat mass (kg)	16±7	44±12	35±12***
Systolic blood pressure (mmHg)	123±17	139±24	127±11$^{\#}$
Diastolic blood pressure (mmHg)	79±12	85±14	81±9
Metabolic status:			
Visceral fat area (cm^2)	56±45	172±79	111±61***
Epicardial adipose tissue (cm^3)	38±15	103±37	84±31**
Liver fat (%)	0.9±0.6	7.1±6.7	3.1±3.3**
Myocardial triglyceride (%)	1.0±0.7	2.1±1.3	1.4±0.8**
Total cholesterol (mmol/l)	4.7±1.0	5.5±1.0	4.7±1.1$^{**\#}$
Triglycerides (mmol/l)	0.98±0.5	1.7±0.7	1.4±0.8$^{**\#}$
Fasting glucose (mmol/l)	4.8±0.3	5.5±0.6	5.0±0.6$^{**\#}$
Insulin (mmol/l)	50±25	111±124	49±25$^{\#}$
HOMA-IR	1.2±0.5	3.0±1.6	1.5±0.9$^{**\#}$
Leptin (ng/ml)	23±12	59±43	30±22$^{**\#}$
Adiponectin (μmol/ml)	19±20	40±52	36±68$^{\#}$
BNP (mmol/l)	7.0±5.0	5.2±2.9	6.5±4.0$^{\#}$

*indicates significant difference from baseline (* $p<0.05$, ** $p<0.01$, *** $p<0.001$); $^{\#}$ (post weight loss intervention only) indicates no significant difference from normal weight controls

3.7 Weight loss and cardiac remodelling

Successful weight loss resulted in positive cardiac remodelling, with LV EDV falling from 161±39ml to 156±37ml ($p=0.001$; table 4.5), and LV mass falling from 115±33g to

106±26g (p=0.006). Ejection fraction remained unchanged, driven by a concomitant reduction in SV (p=0.016) in proportion to the fall in EDV. Change in EDV correlated closely with change in insulin levels (r 0.511, p=0.001) and change in circulating leptin levels (r 0.368, p=0.023), whereas factors such as change in body weight, change in fat mass, fat distribution, and blood pressure were not significant. Mass-volume ratio also fell with successful weight loss (from 0.70±0.11 to 0.68±0.10, p=0.035) indicating that the fall in mass is not only due to the reduction in EDV.

There were no significant changes with weight loss in right ventricular parameters with this small cohort (RV EDV pre 151±65ml, post 149±70ml, p=0.676).

3.7.1 Weight loss and exercise capacity

Despite significant reduction in body weight, and baseline impairment in functional capacity compared to normal weight individuals, weight loss overall did not lead to statistically significant improvements in peak $\dot{V}O_2$ (20±5 ml/kg/min pre vs 20±5 ml/kg/min post, p=1.00; table 4.6), exercise time (9±2 mins pre vs 9±3 mins post, p=0.607) or six minute walk test distance (590±81m vs 611± 82m post, p=0.074).

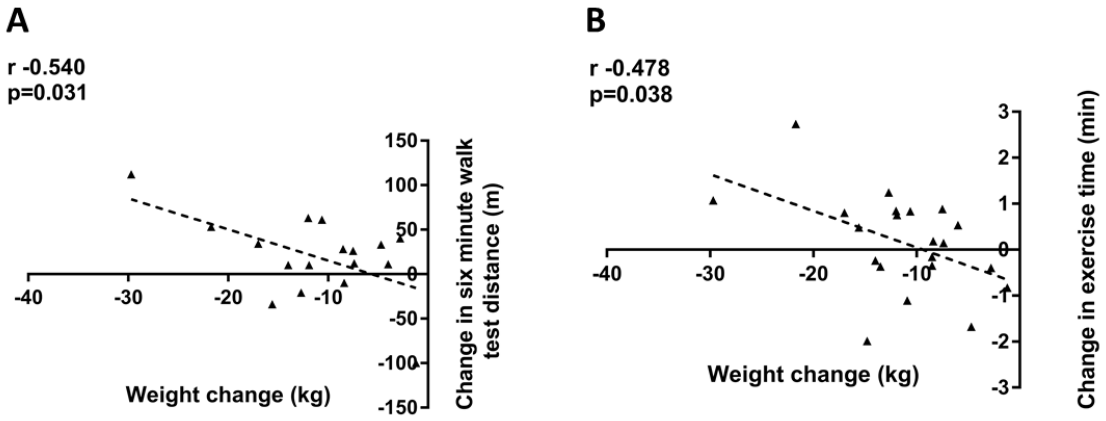


Figure 4.5: Weight change correlates with change in six minute walk test distance (A) and exercise time (B)

However, change in body weight correlated with the change in six minute walk test distance (r -0.555, p=0.009), and change in exercise time (r -0.478, p=0.018).

There were also small but significant deteriorations in percentage of predicted peak $\dot{V}O_2$ achieved, (from 90±20% to 83±19%, p=0.003), and peak $\dot{V}O_2$ indexed for FFM (36±7 ml/kg/min to 33±6 ml/kg/min). Although the improvement in HRR was not significant, HRR post weight-loss was not statistically different from normal controls.

Table 4.6: Impact on cardiac parameters and exercise capacity of successful weight loss

	Normal weight controls n=24	Obese at baseline n=27	Obese post weight loss n=27
Left ventricular parameters:			
End-diastolic volume (ml)	146±27	161±39	154±38$^{**\#}$
End-systolic volume (ml)	55±11	55±17	54±14$^{\#}$
Mass (g)	98±20	116±33	105±26$^{**\#}$
Ejection fraction (%)	63±5	66±5	65±5
E/e'	7.6±1.4	9.0±1.6	7.9±1.8**
Stroke work (l*mmHg)	8±2	10±2	9±2*
Exercise capacity:			
Peak $\dot{V}O_2$ (ml/kg/min)	33±9	20±5	20±5
$\dot{V}O_2$ indexed to fat-free mass (ml/kg/min)	41±10	36±8	33±7**
Exercise time (min)	11±3	9±2	9±3
RER	1.14±0.09	1.13±0.07	1.11±0.07$^{\#}$
VE/$\dot{V}CO_2$	28±4	26±5	29±5$^{**\#}$
Heart rate reserve	0.84±0.12	0.78±0.24	0.86±0.21$^{\#}$
Six minute walk test distance (m)	599±32	592±84	612±82$^{\#}$

*indicates significant difference from baseline (* p<0.05, ** p<0.01, *** p<0.001); $^{\#}$ (post weight loss intervention only) indicates no significant difference from normal weight controls

3.7.2 Weight loss and myocardial energetics

Weight loss caused PCr/ATP to increase (from 1.9±0.3 to 2.1±0.3, p=0.040; figure 4.6A). Weight loss was also associated with a fall in both CK k_f, from 0.17±0.04s^{-1} to 0.12±0.04s^{-1}, p=0.001; figure 4.6B), and ATP delivery (from 1.8±0.6 μmol/g/sec to 1.4±0.6 μmol/g/sec, p=0.018; figure 4.6C). After successful weight loss, all measurements of myocardial energetics were not statistically significant from those recorded in the normal weight cohort (PCr/ATP normal 2.2±0.2, obese post weight loss 2.1±0.3, p= 0.078; CK k_f normal 0.12±0.06s^{-1}, obese post weight loss 0.12±0.04s^{-1}, p=0.742; ATP delivery normal 1.5±0.7 μmol/g/sec, obese post weight loss 1.4±0.6 μmol/g/sec, p=0.987).

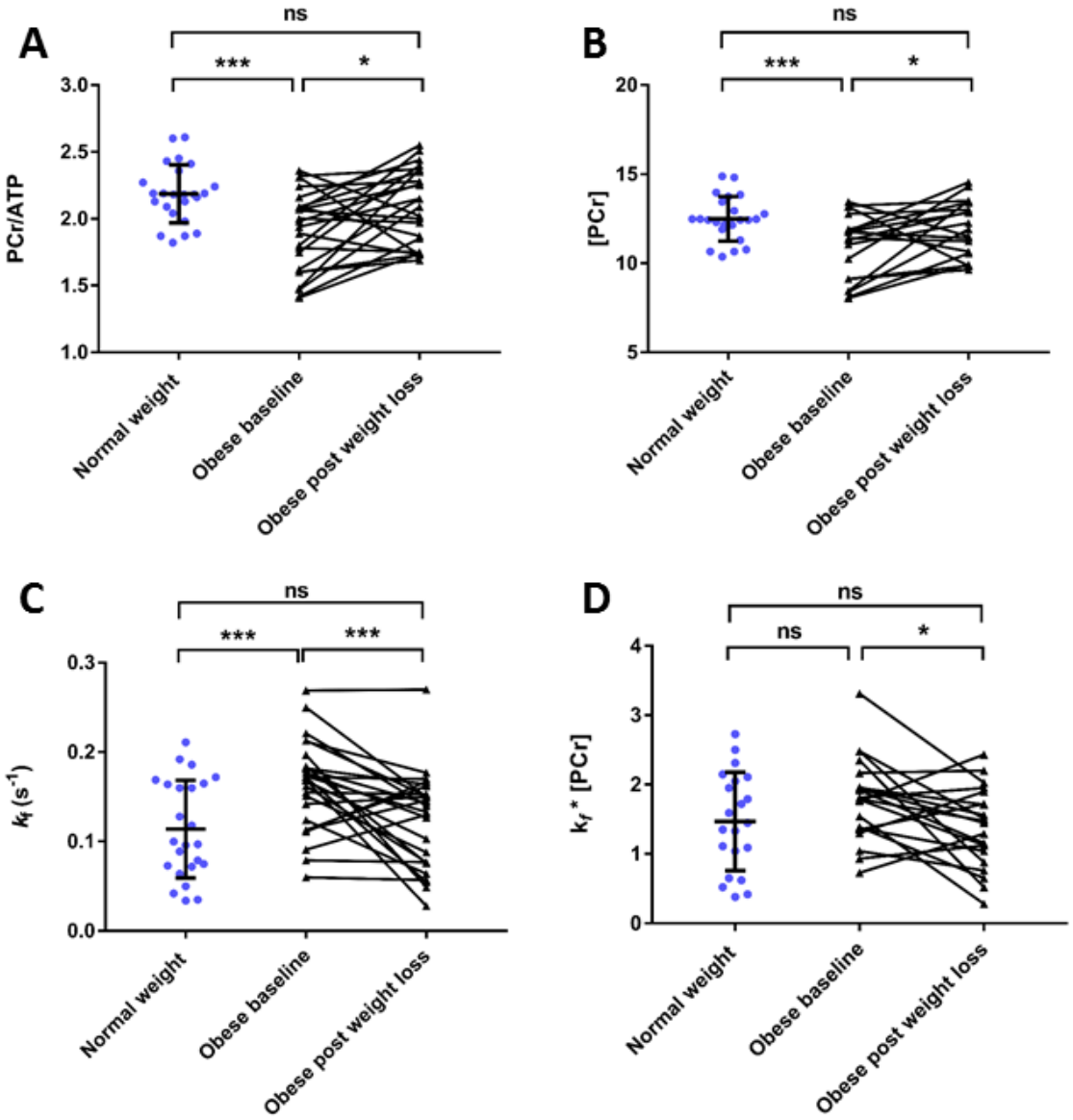


*Figure 4.6: Weight loss in obesity led to normalisation of PCr/ATP (A), PCr concentration (B), creatine kinase forward rate constant (C) and ATP delivery (D). * indicates p<0.05; ** p<0.01; *** p<0.001.*

The change in CK k_f correlated with weight change (r 0.397, p=0.045), visceral fat (r 0.485, p=0.019) and change in insulin (r 0.537, 0.022), but not change in blood pressure, change in LV stroke work or change in leptin concentrations. However, on multiple regression of these parameters, none maintains a statistically significant influence on CK k_f.

Table 4.7: Determinants of the change in creatine kinase k_f with weight loss

	Simple regression		Multiple regression	
	R	p	β	p
Change in visceral fat (%)	0.485	0.019	0.655	0.063
Change in insulin (mmol/l)	0.537	0.022	0.426	0.069
Change in body weight (%)	0.397	0.045	-0.198	0.542

3.7.3 Weight loss and stress energetics

Of the original 14 obese individuals who underwent measurement of dobutamine stress energetics, only 6 proceeded to complete repeat stress testing after the weight-loss intervention (of the others, 4 declined follow-up, 3 declined dobutamine, 1 developed ectopy during infusion). However, these 6 were representative of the rest of the weight loss cohort, in terms of baseline BMI (35±5 kg/m^2 compared to 36±6 kg/m^2, p=0.696) and overall degree of weight loss (-8±7% compared to -9±10%, p=0.818).

Interestingly, even with this small sample size weight loss intervention did lead to changes in the impact of dobutamine stress on CK kinetics. Whereas before weight loss, the obese heart was not able to increase ATP delivery or CK k_f in response to stress, in contrast to the normal heart, following successful weight loss both CK k_f (from 0.11±0.04s^{-1} to 0.19±0.03s^{-1}, p=0.002) and ATP delivery (from 1.4±0.3 μmol/g/sec to 2.7±1.2 μmol/g/sec, p=0.075) increased, albeit with the latter not reaching statistical significance in this number.

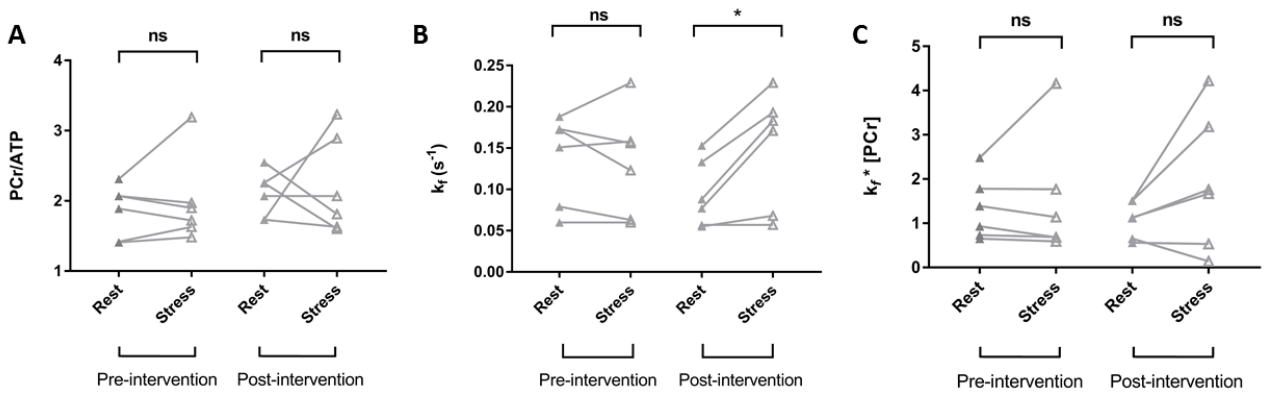


Figure 4.7: Weight loss leads to normalisation of the stress response of creatine kinase k_f (comparison of creatine kinase kinetics before and after weight loss intervention in the same 6 individuals)

4. Discussion

While obesity is in general characterised by normal ejection fraction, it has been established that the myocardial substrate pool for generation of ATP in order to perform contractile work, is depleted in obesity[8]. Therefore it is reasonable to assume that there may be a compensatory mechanism at work, which maintains the rate of ATP delivery in the face of diminished [PCr]. There is some animal[213] and tissue[214]data to support a hypothesis that this compensatory mechanism is via elevated reaction rate of creatine kinase, but this is yet to be investigated in humans. Here I have demonstrated for the first time in humans, the impact of increasing body fat mass on phosphotransfer rates via CK in the obese myocardium.

I have confirmed that PCr/ATP, analysed non-invasively with ^{31}P-MRS and reflecting [PCr], is depleted in obesity, even at rest and in the absence of traditional measures of impaired systolic function. This apparent deficit in substrate pool is likely to be related to the imbalance between increased left ventricular stroke work in the face of sympathetic and neuroendocrine stimulation, and decreased efficiency of cardiac metabolism[84] due in part to enforced utilisation of FFAs as the sole substrate for respiration. A reduced PCr/ATP ratio has been linked with clinical outcomes in heart failure[88] and has been postulated to be an intrinsic component of the final pathway leading to functional decline in both heart failure[69], and the natural process of ageing.

The majority of previous studies of *in vivo* human myocardial creatine kinase have demonstrated a reduced rate of activity in the direction of ATP generation, in processes including coronary disease[94], dilated cardiomyopathy[93] and hypertrophic cardiomyopathy[216]. In contrast, in this obese cohort without systolic dysfunction as

measured by traditional methods, I have demonstrated increased forward rate constant of the enzyme, in proportion to body fat mass. The overall ATP delivery rate, or CK flux, is not statistically different to the normal weight population, but there is a trend towards increased energy delivery. This may suggest that the enzyme rate is increased in order to compensate for the depleted substrate pool, and hence ATP delivery is maintained. In line with this, there have been studies showing the same pattern in obese/diabetic animal models[213] and in skeletal muscle[214]. However, this is the first time that a potentially compensatory increase in CK enzyme kinetics has been demonstrated *in vivo* in the obese myocardium.

However, the fact that the increase in CK k_f seems to be in proportion to the increased ventricular stroke work seen at rest in obesity, might in contrast indicate that it is the haemodynamic demand driving the enzymatic rate constant, in combination with the depleted substrate pool, rather than an intrinsic effect mediated by obesity. Consistent with this hypothesis is the fact that, in obesity, CK k_f fails to augment further in response to catecholamine stress, as is the case in normal weight individuals, suggesting that in obesity the enzyme is constitutively functioning at a rate closer to its maximum capacity at rest, with less reserve available to increase energy delivery in response to demand. The return of capacity to augment k_f rate with stress following weight loss supports this hypothesis, albeit in small numbers.

This energetic limitation, as well as the fact that LV ejection fraction increases to a lesser degree with stress than in normal weight, might help to explain why exertional symptoms are so prevalent in obesity. One of the diagnostic criteria for a diagnosis of HFpEF is worsening diastolic dysfunction with exercise. As diastolic function is more

sensitive to energetic deficit than systolic, the findings in this chapter provides a potential energetic explanation for the exertional element of HFpEF.

I would suggest that these compensatory energetic changes at rest, at the cost of reduced ability to cope with stress, are perhaps an early feature of the development of heart failure, before continued demand outstripping supply leads to eventual fall in energy generation, and decompensation of contractile mechanisms.

4.1 Regulation of creatine kinase

The mechanism of this increase in enzyme activity is unclear, and obviously not addressed directly by this non-invasive study. However, it has previously been shown that pathologies such as hypertrophy and heart failure are associated with an alteration in the normal expression of creatine kinase isoenzymes[217], with a change back towards a foetal profile of expression with increased CK-B but reduced CK-M. In heart failure, these changes are reversed with implantation of a left ventricular assist device[218]. One potential hypothesis is that obesity is associated with an abnormal pattern of isoenzyme expression, which may explain the altered reaction kinetics seen.

However, it has also been demonstrated that there are significant post-transcriptional changes which perhaps play an even more significant role in determining enzyme activity particularly in terms of rapid changes in activity in response to dynamic energetic demand. Myocardial creatine kinase is one of the downstream targets of protein kinases including PKC[219] and AMPK[220], and phosphorylation reduces its activity. Lin et al[219] demonstrated that although CK-M expression is indeed downregulated in diabetic hearts, the degree of phosphorylation of the enzyme is reduced to an even greater degree,

leading overall to increased enzyme activity. The mechanisms by which creatine kinase is regulated are clearly complex, and so far little investigated in obesity.

4.2 Creatine kinase in obesity

Obesity is associated with a range of pathological changes which have, independently, been implicated in modulating creatine kinase expression and/or activity, including increased sympathetic activity[221], generalised inflammation[222], and insulin resistance[223]. However, the results described in this chapter suggest that increased fat mass is associated with the increased CK k_f, and so this may be related to adipokines secreted by the fat tissue itself. Increased levels of circulating leptin are associated with both hypertrophy and insulin resistance, and the hormone has been implicated[195] in activating AMP kinase, a key determinant of substrate utilisation and energy output[78]. It has also been shown that the increase in AMP kinase activity in response to depleted energy stores is blocked in leptin resistance[224], which may affect creatine kinase activity. These data confirm the possibility of an interaction between CK activity and leptin concentrations, with a positive correlation seen between the two. Additionally, adiponectin has been found to be implicated in AMPK activation[225].

5. Conclusions

Here I demonstrate for the first time that obesity is associated with increased activity of the creatine kinase enzyme in human myocardium, and postulate that this may be a compensatory mechanism to address the haemodynamic, metabolic and energetic challenges of the obese state. Increased creatine kinase kinetics have been associated with improved outcomes, and it is feasible that the improved survival seen with obesity in cardiac disease is related to compensatory modulation of this energy-delivery pathway. There are numerous potential mechanisms by which obesity may influence creatine kinase activity, and these are exciting targets for further study.

Chapter Five

The Impact of Obesity in Heart Failure

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1. Background

Despite the fact that obesity is a key risk factor for developing heart failure, both with preserved (HFpEF) and reduced (HFrEF) left ventricular systolic function[2-4], there exists an apparently paradoxical relationship between survival in established heart failure and BMI[14], which suggests that increasing body size is associated with improved survival. Given the numerous physiological challenges invoked by increasing body fat mass and its metabolic complications, this is surprising and rather counterintuitive.

A number of potential explanations for this phenomenon exist[166], including statistical bias on a population level, earlier presentation in obese individuals, or obesity merely acting as a marker corresponding to a different population. However, one hypothesis is that there is a protective difference in the physiology of obesity which is beneficial in heart failure, and this is worthy of further investigation. Additionally, there is evidence that many of the cardiovascular complications of obesity are mediated via a dysfunctional RAAS signalling cascade[226], and hence blockade of this system with pharmacological heart failure treatment may have an additional synergistic impact compared to heart failure in normal weight individuals.

Myocardial energetic status in heart failure has been linked with prognosis. Depleted myocardial energy stores as represented by PCr/ATP, were demonstrated to be

associated with worse survival[88], and postulated to be a common final pathway in the progression towards heart failure[69]. PCr/ATP has been demonstrated to also be lower in the obese state in the absence of heart failure[8], reiterated in the previous chapter.

Similarly, reduced activity of myocardial creatine kinase, a key enzyme which facilitates the ability of PCr to act as both spatial and temporal buffer of ATP in the myofibril, has been linked with both worsening symptoms and deterioration in prognosis in heart failure[96]. However, in contrast to PCr/ATP, I have demonstrated in the previous chapter that the forward rate constant of the creatine kinase reaction is in fact higher in the obese state than normal weight individuals. This raises a fascinating question – whether obesity confers a favourable energetic state in terms of with higher creatine kinase kinetics in heart failure. This could then enable preservation of ATP delivery and provide a plausible physiological basis for the obesity paradox.

In order to address this hypothesis, I first studied obese individuals with established dilated cardiomyopathy, and compared the results to normal weight individuals with heart failure in order to investigate whether the elevated CK rate constant was also seen in heart failure. I then related these results to BMI-matched individuals with normal systolic function to assess the impact of heart failure on creatine kinase kinetics. As exertional symptoms are almost universally seen in heart failure, as well as obesity in isolation, I also examined the impact of catecholamine stress on myocardial energetics in these groups, comparing any changes to cardiac function and exercise capacity.

2. Methods

For detailed methods see Chapter Two.

In brief, the study was approved by the local ethics board (NRES reference 14/SC/004). 43 individuals with established dilated cardiomyopathy (EF 25-45%) were recruited from heart failure clinics at a tertiary referral cardiology centre. 16 were normal weight (BMI 18.0-25.0 kg/m^2) and 27 obese (BMI>30 kg/m^2). All signed written consent for the study investigations.

Results were compared to 72 age- and BMI-matched volunteers with normal systolic function, described in Chapter Four.

2.1 Inclusion criteria

Participants were excluded if they had any history of coronary artery disease or severe valvular disease. Additionally, uncontrolled hypertension, atrial fibrillation or diabetes mellitus were exclusion criteria, as was any contraindication to MR scanning.

2.2 Anthropomorphic and biochemical assessment

Height, weight and body composition were measured using digital scales with bio-impedance analysis. Fasting venous blood was taken and total cholesterol, triglycerides, glucose, insulin and BNP were analysed by the Oxford University Hospitals clinical biochemistry laboratory according to standardised protocols. In addition, leptin and adiponectin were analysed using commercially available ELISA kits.

2.3 Magnetic resonance imaging

All MRI was performed at 3T (Siemens Tim Trio). Abdominal visceral fat, hepatic fat fraction, cardiac structure and systolic function, and myocardial triglyceride content using ^{1}H spectroscopy were acquired as per Chapter Two.

2.3.1 Phosphorus spectroscopy

^{31}Phosphorus spectroscopy was performed on the same 3T system described above. Creatine kinase k_f was measured using a TRiST sequence as developed by Schar et al[183].

The relative ratio of PCr to γ-ATP peaks in the fully relaxed spectrum is used to generate PCr/ATP ratio, and literature values for [ATP][93] used to calculate [PCr]. The overall ATP delivery, or CK flux, was then calculated as $k_f^{\star}$ [PCr].

In order to measure the effect of catecholamine stress on CK k_f, the STreST protocol was acquired under low-dose dobutamine stress according to the protocol outlined in Chapter Two.

^{31}P-MRS data was analysed post hoc using AMARES analysis software within Matlab.

2.4 Echocardiography

Echocardiography was performed on a Philips Epiq system, to determine diastolic function; pulse wave velocity was measured at mitral valve inflow and tissue Doppler at lateral and medial mitral valve annulus.

2.5 Cardiopulmonary exercise testing

Volunteers underwent cardiopulmonary exercise testing at each visit, with measurement of maximal capacity on bicycle ergometer, and submaximal capacity on six minute walk test assessment.

2.6 Statistical analysis

Statistical analysis was performed using commercial software (SPSS, Chicago). All data is presented as mean ± standard deviation. Normality of distribution was confirmed before determination of statistical significance, either by Chi-squared tests in the case of categorical data, or student's t tests for continuous data (paired or independent where relevant). Non-parametric data was analysed with the Mann Whitney U test, or Kruskal Wallis test, with paired data analysed using the Wilcoxon Rank test. Pearson's correlation models and simple linear regression were used where relevant. Values of $p<0.05$ were considered as statistically significant.

3. Results

3.1 The impact of obesity in heart failure

3.1.1 Baseline anthropomorphic characteristics

27 obese individuals (mean BMI 37±5 kg/m^2) with established dilated cardiomyopathy (EF 25-45%) were recruited, and results compared to 16 normal weight individuals with established dilated cardiomyopathy (mean BMI 23±2 kg/m^2, $p<0.001$ compared to study group).

The two groups (see table 5.1) were well-matched for age (normal weight 59±16 years, obese 57±11 years, $p=0.748$) and sex (63% male in both groups, $p=0.976$). The obese group had significantly higher baseline body fat mass (46±14kg vs 17±8kg in the normal weight group, $p<0.001$), with evidence of ectopic fat deposition in the liver (hepatic fat 5.0±4.0% compared to 1.7±1.4%, $p=0.002$). Interestingly, myocardial triglyceride levels were not statistically different (1.3±0.7% in the obese group, vs 1.4±1.1% in normal weight, $p=0.741$).

Table 5.1: Baseline cohort characteristics compared to normal weight heart failure

	Normal weight heart failure n=16	Obese heart failure n=27	p
Age (years)	59±16	57±11	0.748
Male (% of total)	10 (63)	17 (63)	0.976
Body weight (kg)	67±9	109±20	**<0.001**
Body mass index (kg/m^2)	23±2	37±5	**<0.001**
Body fat mass (kg)	17±8	46±14	**<0.001**
Systolic blood pressure (mmHg)	125±25	129±16	0.518
Diastolic blood pressure (mmHg)	72±18	81±14	0.154
Metabolic status:			
Abdominal visceral fat (cm^2)	74±29	212±109	**<0.001**
Hepatic fat (%)	1.7±1.4	5.0±4.0	**0.002**
Myocardial triglyceride content (%)	1.3±0.7	1.4±1.1	0.741
Total cholesterol (mmol/l)	5.0±1.1	4.8±1.0	0.460
Triglycerides (mmol/l)	1.3±0.6	2.1±1.2	**0.008**
Fasting glucose (mmol/l)	5.2±0.6	6.4±2.2	**0.025**
Fasting insulin (mmol/l)	64±34	142±78	**<0.001**
HOMA-IR	3.1±2.0	8.9±7.5	**0.002**
BNP (pg/ml)	39±43	21±25	0.106
Drug therapy:			
ACE inhibitor	13 (81)	17 (65)	0.269
Angiotensin receptor blocker	2 (13)	7 (26)	0.269
Beta blocker	11 (69)	22 (82)	0.224
Aldosterone antagonist	8 (50)	11 (41)	0.627
Diuretic	6 (38)	11 (41)	0.130

3.1.2 Baseline metabolic and cardiovascular characteristics

Blood pressure was similar between the two groups, as was total cholesterol. However, circulating triglycerides were higher in obesity (2.1±1.2 mmol/l vs 1.3±0.6 mmol/l in normal weight, p=0.008). The obese group was significantly more insulin resistant, with higher fasting glucose (6.4±2.2 mmol/l vs 5.2±0.6 mmol/l, p=0.025), and HOMA-IR (8.9±7.5 vs 3.1±2.0, p=0.002). BNP levels were similar between the two groups, although with the suggestion of a trend towards lower values in obesity (21±25 pg/ml compared to 39±43 pg/ml, p=0.066 Mann Whitney U test).

There was no significant difference between the groups in terms of pharmacological therapy. Both were well-treated, with similar numbers taking ACE inhibitors (89% in obese HF, 92% of normal weight HF, p=0.845), and beta blockers (69% normal weight HF, 82% obese HF, p=0.224).

The two groups were well-matched for cardiac parameters, with no significant differences between LV end-diastolic volume, stroke volume and ejection fraction (normal weight 38±6% vs obese 41±7%, p=0.147; table 5.2).

Table 5.2: Baseline cardiovascular parameters and exercise capacity

	Normal weight heart failure n=16	Obese heart failure n=27	p
Left ventricular parameters:			
End-diastolic volume (ml)	210±47	227±58	0.337
End-systolic volume (ml)	143±61	136±46	0.673
Stroke volume (ml)	86±32	91±20	0.538
Ejection fraction (%)	38±6	41±7	0.147
Mass (g)	144±41	163±38	0.140
LV stroke work (l*mmHg)	5.0±3.1	4.7±1.9	0.689
Exercise capacity:			
Peak $\dot{V}O_2$ (ml/kg/min)	25±8	17±4	**0.007**
Peak $\dot{V}O_2$ adjusted for FFM (ml/kg/min)	33±9	29±6	0.152
Maximum HR (bpm)	145±29	137±24	0.358
RER	1.1±0.1	1.1±0.1	0.209
VE/$\dot{V}CO_2$	32±6	29±5	0.220
Percentage predicted $\dot{V}O_2$ achieved (%)	92±24	69±17	**0.049**
Exercise time (min)	9±2	6±2	**0.012**
Six minute walk test distance (m)	574±102	500±104	**0.008**

3.1.3 Impact of obesity and heart failure on exercise capacity

Despite very similar metabolic and cardiac functional parameters, the obese group were significantly limited in terms of whole body functional capacity (peak $\dot{V}O_2$ 17±4 ml/kg/min compared to 25±8 ml/kg/min normal weight HF, p=0.007), total exercise time to reach peak $\dot{V}O_2$ (6±2 mins as opposed to 9±2 mins, p=0.012), and six minute

walk test distance (500±104m compared to 574±102m, p=0.008). There was no difference between maximum achieved heart rate, RER or ventilatory capacity ($VE/\dot{V}CO_2$) which was within the normal range for both groups.

If functional capacity in both heart failure groups is compared to the BMI-matched individuals without heart failure, previously described in Chapter Four, it is interesting to note that in both normal weight and obese individuals, heart failure is associated with lower peak $\dot{V}O_2$ than those without heart failure (see figure 5.1).

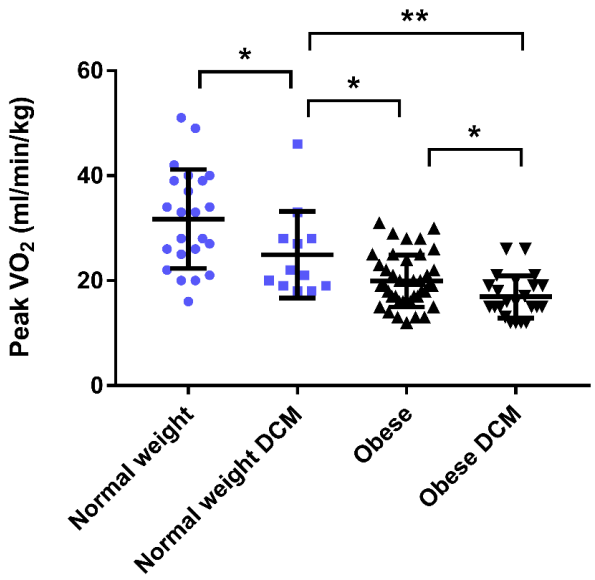


*Figure 5.1: Exercise capacity in normal weight (in blue) and obese (in black) groups with and without heart failure. * indicates p<0.05; ** p<0.01.*

3.2 Myocardial energetics in obese heart failure

3.2.1 PCr/ATP

In the established heart failure groups, there was no difference conferred by increasing BMI on resting PCr/ATP (obese heart failure 1.7±0.2 vs normal weight heart failure 1.7±0.2, p=0.480) or [PCr] calculated using reference values for [ATP] (obese 9.0±1.2 μmol/g vs normal weight 8.7±0.9 μmol/g, p=0.480).

When results are compared to BMI-matched individuals with normal systolic function, PCr/ATP was lower in normal weight heart failure (heart failure 1.7±0.2 vs controls 2.2±0.2, p<0.001), reflecting lower [PCr] (heart failure 8.7±0.9 μmol/g compared to 12.5±1.2 μmol/g, p<0.001). In a similar fashion, PCr/ATP was lower in obese heart failure than obese individuals with normal systolic function (heart failure 1.7±0.2 vs controls 1.9±0.3, p=0.031), as was [PCr] (heart failure 10.8±1.7 μmol/g compared to 12.5±1.2 μmol/g, p<0.001).

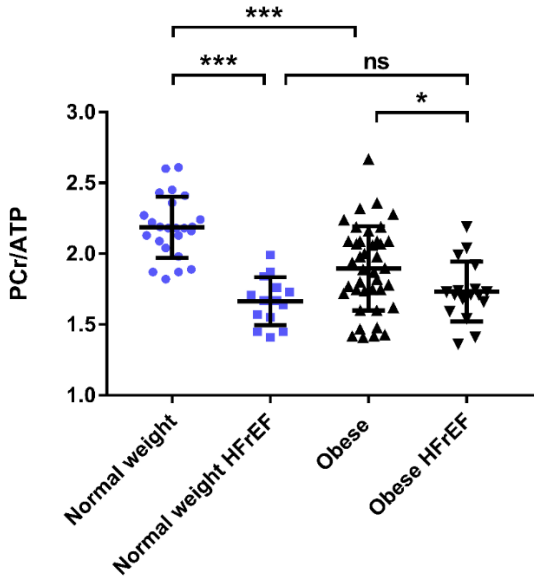


*Figure 5.2: PCr/ATP is lower heart failure than controls in normal weight (blue) but not obese (black). * indicates p<0.05, *** indicates p<0.001.*

Presented in the previous chapter, in individuals with normal systolic function, there is a negative correlation between BMI and PCr/ATP (r -0.453, p<0.001); this is lost in heart failure (p=0.615). Again only in normal systolic function does PCr/ATP associate with peak $\dot{V}O_2$ (r 0.508, p<0.001). The correlation between PCr/ATP and ejection fraction which exists in normal weight individuals with normal systolic function is also lost in heart failure (p=0.371).

3.2.2 Creatine kinase kinetics

In a similar fashion to the difference demonstrated in normal systolic function, there was a significant difference in CK k_f between obese heart failure and normal weight heart failure – in other words, obesity is associated with a significantly higher reaction rate than normal weight (obese heart failure 0.17±0.08s^{-1} vs normal weight heart failure 0.08±0.06 s^{-1}, p<0.001). Similarly, creatine kinase k_f correlated positively with increasing body fat in individuals with heart failure (figure 5.3).

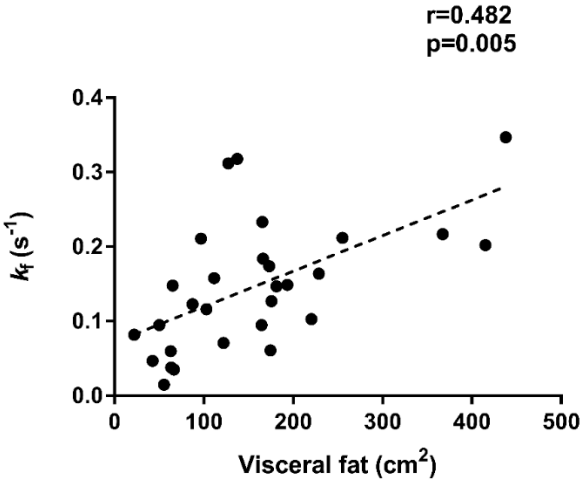


Figure 5.3: The forward rate constant of the creatine kinase reaction (k_f) correlates with visceral fat area in heart failure

However, there was no difference between creatine kinase forward rate constant at rest in normal weight controls and normal weight heart failure (0.11±0.05 s^{-1} vs 0.08±0.06 s^{-1}, p=0.128), or between obese controls and obese heart failure (0.16±0.05 vs 0.17±0.08, p=0.629),

As discussed in Chapter Four, whereas in normal systolic function the difference in creatine kinase k_f may have been partly explained by the difference in rate pressure product, there was no significant difference in resting RPP (HR*systolic blood pressure) in the heart failure groups (normal weight 7626±1561 mmHg*bpm, obese 9208±2666

mmHg*bpm, p=0.157). Additionally, if k_f is indexed to RPP, the difference between the group persists (p=0.022).

3.2.3 ATP delivery

Literature values for [ATP][93] in normal cardiac function and heart failure were used together with the measured PCr/ATP and creatine kinase k_f to generate a value for ATP delivery. The elevated creatine kinase k_f in obesity means that, despite the depleted PCr/ATP measured in both heart failure groups, in obese heart failure, resting ATP delivery rates are higher than in normal weight heart failure (obese heart failure 1.6±0.8 μmol/g/sec vs normal weight heart failure 0.7±0.6 μmol/g/sec, p=0.002, figure 5.4).

When comparing normal weight heart failure to normal weight controls, the depleted PCr/ATP in normal weight heart failure means that overall ATP delivery is lower (normal weight control 1.4±0.7 μmol/g/sec vs normal weight heart failure 0.7±0.6 μmol/g/sec, p=0.004). However, in obese heart failure, the elevated creatine kinase k_f compensates for low PCr/ATP, allowing the overall ATP delivery value to remain similar to that seen in normal systolic function despite PCr/ATP depletion (obese control 1.8±0.6 μmol/g/sec vs obese heart failure 1.6±0.8 μmol/g/sec, p=0.348).

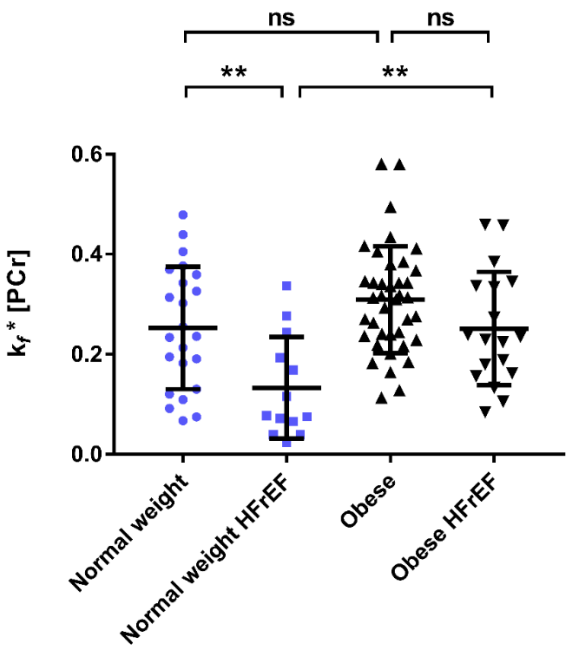


*Figure 5.4: Flux through the creatine kinase reaction is lower in heart failure, whether normal weight (blue) or obese (black). ** indicates p<0.01, *** p<0.001*

3.3 The impact of dobutamine stress in normal weight heart failure

Of the normal weight heart failure volunteers, 7 underwent dobutamine infusion, to a target heart rate of 68±2% of maximum, for a period of 24±3 minutes during which creatine kinase kinetics were re-assessed. These parameters were not statistically different from the control group (all p>0.05, table 5.3).

Although the increase in ejection fraction was in effect higher in heart failure than BMI matched controls (relative increase of 40±16% compared to 28±10%), reflecting lower resting values, this was not statistically significant (p=0.095). In addition, the increase in stroke work (stroke volume*mean arterial pressure), was no different (+76±40% compared to +110±72%, p=0.404), and the increase in RPP with stress was lower (+96±43% compared to +159±57%, p=0.049).

Table 5.3: Haemodynamic response to stress in normal weight cohorts

	Normal weight controls n=20	Normal weight heart failure n=7	p
Total duration dobutamine (mins)	24±3	24±3	0.852
Resting heart rate (bpm)	57±7	65±10	**0.041**
Stress heart rate (bpm)	113±8	111±8	0.455
Peak heart rate (% maximum)	64±4	66±5	0.294
Resting systolic blood pressure (mmHg)	121±17	134±20	0.115
Maximum systolic blood pressure (mmHg)	151±14	145±14	0.311
Resting LV ejection fraction (%)	63±4	41±7	**<0.001**
Stress LV ejection fraction	80±7	57±9	**<0.001**
Resting RPP (mmHg*bpm)	6899±1292	8080±1553	0.092
Stress RPP (mmHg*bpm)	17264±1793	16358±1169	0.298
Relative increase in RPP (%)	159±57	96±43	**0.049**
Resting LV stroke work (l*mmHg)	4.3±1.3	3.9±0.9	0.485
Stress LV stroke work (l*mmHg)	8.1±2.2	7.0±2.4	0.405

3.3.1 Myocardial energetic response to stress

Whereas in the normal weight control group, dobutamine stress led to no significant change in PCr/ATP (rest 2.2±0.2 vs stress 2.2±0.4, p=0.463), in heart failure a fall in PCr/ATP was observed (from rest 1.7±0.2 to stress 1.3±0.5, p=0.034). However, no change in either creatine kinase reaction rate (rest 0.08±0.07s^{-1} to stress 0.08±0.07s^{-1}, p=0.958) or overall ATP delivery (rest 0.7±0.6 μmol/g/sec to stress 0.5±0.5 μmol/g/sec, p=0.334) was measured with stress, in contrast to the significant increase seen in normal heart controls (CK k_f rest 0.13±0.06 s^{-1} to stress 0.24±0.12 s^{-1}, p<0.001; ATP delivery 1.6±0.7 μmol/g/sec to stress 3.0±1.7 μmol/g/sec, p=0.001).

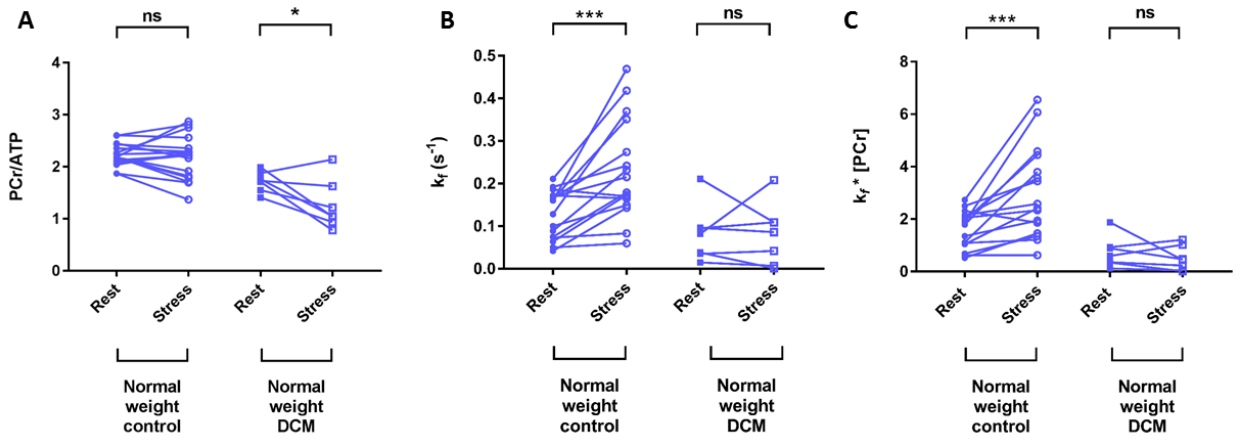


Figure 5.5: Dobutamine stress leads to a fall in PCr/ATP in normal weight heart failure (A), whereas no change is induced in creatine kinase rate constant (B) or overall ATP delivery (C) in contrast to the normal heart. ⋆ indicates p<0.05, ⋆⋆⋆ indicates p<0.001.

In a similar fashion to the normal heart, there is a positive correlation in normal weight heart failure between the amount by exercise capacity (peak $\dot{V}O_2$) and the change in creatine kinase k_f with dobutamine stress (r 0.914, p=0.004).

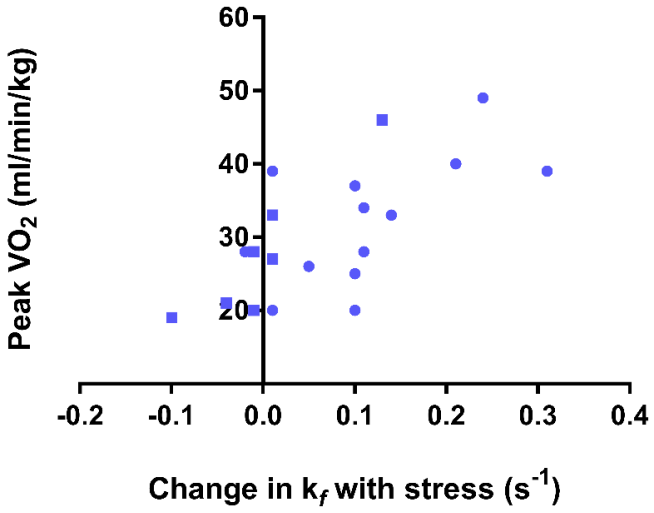


Figure 5.6: The change in creatine kinase forward rate constant (k_f) correlates with peak $\dot{V}O_2$ in both normal weight (circles (r 0.604, p=0.029)) and normal weight heart failure (squares (r 0.914, p=0.004))

3.4 The impact of dobutamine stress in obese heart failure

Of the obese heart failure group, 15 underwent dobutamine stress, with no significant differences in any measured parameter of the stress test compared to the normal weight heart failure group (all p>0.05). Compared to the control group, there was no difference in heart rate achieved (controls 64±9% vs heart failure 68±11%, p=0.299) or length of infusion (controls 25±2 mins vs heart failure 24±3 mins, p=0.792). However, similar to the response seen in the normal weight heart failure group, there was lower relative increase in RPP (controls 127±39% vs heart failure 93±40%, p=0.033) and maximum blood pressure (controls 168±19 mmHg vs heart failure 145±16 mmHg, p=0.001).

Table 5.4: Haemodynamic response to stress in obese cohorts

	Obese controls n=20	Obese heart failure n=15	p
Total duration dobutamine (mins)	25±2	24±3	0.792
Resting heart rate (bpm)	64±9	68±11	0.359
Stress heart rate (bpm)	114±10	108±8	0.087
Peak heart rate (% maximum)	63±16	68±7	0.299
Resting systolic blood pressure (mmHg)	133±15	127±14	0.233
Maximum systolic blood pressure (mmHg)	168±19	145±16	**0.001**
Resting LV ejection fraction (%)	65±3	42±7	**<0.001**
Stress LV ejection fraction (%)	78±6	57±11	**<0.001**
Resting RPP (mmHg*bpm)	8590±1595	8146±1387	0.453
Stress RPP (mmHg*bpm)	19071±2085	15674±1891	**<0.001**
Relative increase in RPP (%)	127±39	93±40	**0.033**
Resting LV stroke work (l*mmHg)	5.9±2.2	4.1±1.7	**0.021**
Stress LV stroke work (l*mmHg)	12.0±4.7	6.7±2.8	**0.001**

Again, similar to the normal weight heart failure group, the relative increase in ejection fraction with stress was higher in obese heart failure than in normal systolic function (relative increase of 40±29% compared to 20±12%, p=0.026). Additionally, although there was a significant increase in LV stroke work with stress in both groups (obese heart failure from 4.1±1.7 l*mmHg to 6.7±2.8 l*mmHg, p=0.028; obese controls from 5.9±2.2 l*mmHg to 12.0±4.7 l*mmHg, p<0.001), the magnitude of increase is not statistically different in obese controls than obese heart failure (control increase by 111±70%, heart failure by 78±66%, p=0.199).

3.4.1 Myocardial energetic response to stress

In a similar fashion to the normal weight heart failure group, in obesity, dobutamine stress led to a significant fall in PCr/ATP (from 1.7±0.3 to 1.0±0.2, p<0.001), in contrast to the control group with normal systolic function where there was no significant change (figure 5.6). While there was no numerically significant fall in creatine kinase k_f (0.18±0.07s^{-1} to 0.15±0.09s^{-1}, p=0.142), overall ATP delivery fell (from 0.33±0.16 to 0.15±0.11, p=0.001). There is no direct correlation between the energetic changes observed with stress, and any cardiac functional parameter.

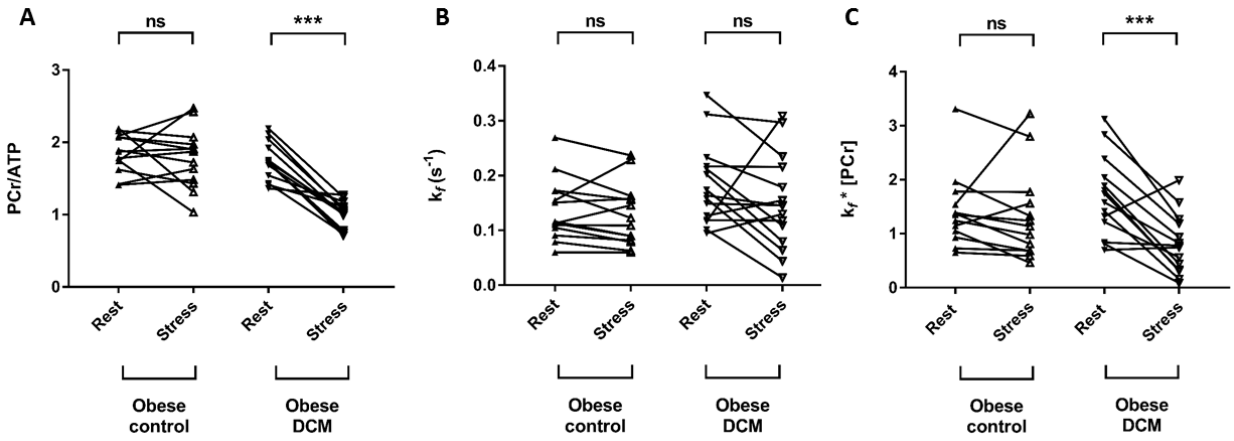


*Figure 5.6: Dobutamine stress in obese heart failure is associated with a fall in PCr/ATP (A), no change in creatine kinase reaction rate (B), but an overall fall in ATP delivery (C). ** indicates p<0.01, *** p<0.001.*

In contrast to the relationship observed in normal weight individuals (peak $\dot{V}O_2$ r 0.622, p=0.031), and in a similar fashion to obese individuals with normal ejection fraction (r -0.147, p=0.824), there is no correlation between the change in creatine kinase k_f with dobutamine stress, and peak $\dot{V}O_2$ (r 0.072, p=0.824).

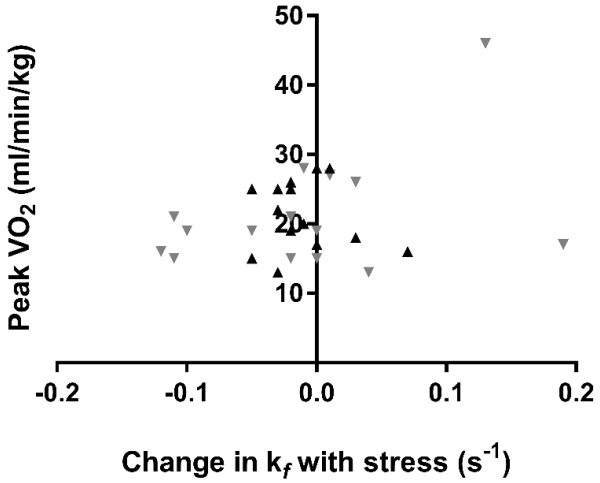


Figure 5.7: The relationship between change in creatine kinase kinetics with dobutamine stress and peak $\dot{V}O_2$ in obesity (grey triangles indicate DCM (r 0.072, p=0.824) and black triangles normal ejection fraction (r -0.147, p=0.616)).

4. Discussion

While obesity is one of the most common risk factors for the development of heart failure, the existence of the obesity paradox raises the question as to whether there is some kind of protective influence of the obese state, which improves prognosis in established heart failure. While a number of studies, including this thesis, have demonstrated depleted energetic stores in terms of PCr/ATP in obesity[8], in the previous chapter I established that in fact the reaction rate of myocardial creatine kinase is greater in obesity with normal systolic function, when compared to normal weight individuals, and therefore maintaining ATP delivery rates. This parameter has been linked to prognosis in heart failure[96], and underlines the fact that relatively little is known about myocardial energy transfer networks in heart failure, particularly in the context of increasing body fat mass and metabolic abnormalities. In this study, I have demonstrated that a similar difference can be measured in established heart failure – namely that the reaction rate of myocardial creatine kinase is higher in obesity than normal weight individuals, leading to greater ATP delivery despite baseline depletion of energetic stores. Further work will be needed to determine the significance of this finding in terms of prognosis, our understanding of the underlying pathophysiology of heart failure, and most importantly the implications for the management of obesity-related heart failure.

4.1 Myocardial energetics in heart failure

This study confirmed the findings of others, in terms of energetic stores as measured by PCr/ATP being depleted in both heart failure[87, 88] and obesity in the absence of heart failure[8]. However, for the first time, a spectrum of energetic depletion appears, with PCr/ATP in an obese population with a degree of insulin resistance, resting diastolic

dysfunction and exercise limitation being significantly lower than a normal weight control population, and yet individuals in either weight category with systolic dysfunction, having an even lower baseline PCr/ATP.

In contrast to both PCr/ATP and to previously published studies[93], there was no measurable difference in creatine kinase k_f in heart failure, either between normal weight or obese cohorts. This may reflect differences in resting cardiac function between the studies (in Weiss et al[93], median LVEF was 20%, whereas here the mean was 40%). However, in a similar picture to findings in the previous chapter, obese individuals with heart failure had higher rates of creatine kinase activity at rest than normal weight individuals with heart failure, in proportion to body fat. Using both parameters together to understand overall resting ATP delivery, it is possible to discriminate between normal weight controls and normal weight heart failure, with lower creatine kinase flux generated in heart failure due to significantly depleted [PCr] and numerically lower rate of creatine kinase activity. However, net creatine kinase flux in obese heart failure is not statistically different from those with normal systolic function, with the increased reaction rate of creatine kinase compensating for lower [PCr]. Therefore, overall myocardial ATP delivery is higher at rest in obese heart failure than normal weight heart failure, despite similar resting stroke work and RPP.

Given that ATP flux via the creatine kinase system has been demonstrated to hold more sensitive prognostic information than ejection fraction[96], this is potentially of significance. The potential mechanisms mediating the elevation in resting CK k_f rate are touched on previously, but include increased haemodynamic demand on the heart by increased blood volume and metabolic demand of increased body size, relative overactivity of the

sympathetic nervous system leading to activation of intracellular signalling cascades and alteration in activity of intracellular kinases, the impact of intracellular insulin resistance and the altered neurohormonal environment created by increased whole body adipose tissue, and epicardial fat volume and quality.

4.2 The impact of stress on myocardial energetics in heart failure

Here, the data demonstrates that the resting energetic deficit found in heart failure is exacerbated by a period of dobutamine stress, representing in a reproducible experimental fashion a period of increased physiological workload. In both normal weight and obese heart failure groups, the PCr/ATP falls further with dobutamine stress, suggesting that the myocardial use of ATP outstrips the ability of PCr to temporally buffer demand, as well as the metabolic reserve of the tissue. Normally, the capacity of the myocardium to continually generate energy greatly exceeds demand, as is seen in the normal heart where PCr/ATP is not compromised by moderate increase in workload. However, in conditions such as heart failure, alterations in baseline substrate metabolism and inability to substrate switch when required, leads to a reduction in this metabolic reserve, and ultimately in a fall in PCr/ATP.

In contrast to the response to increased workload in the normal heart, where creatine kinase rate constant increases with stress, in normal weight individuals with heart failure, this response is not seen. Therefore overall ATP delivery does not increase with stress, whereas a 1.8-fold increase is seen in the normal heart.

In obesity however, the response to pharmacological stress in the normal heart is an inability to increase the creatine kinase rate constant, and a similar finding is observed in obese heart failure. Combined with the fall in PCr/ATP with stress, the impact of

increased workload in obese heart failure is a fall in overall ATP delivery. One potential explanation for this is the fact that the enzyme reaction rate at baseline is higher, leading to diminished reserve in capacity to cope with increased requirements in the face of depleted substrate pool.

It is interesting to observe that whole body maximal exercise capacity, as measured by peak $\dot{V}O_2$ and limited by cardiovascular capacity, correlates with the change in creatine kinase activity with dobutamine stress, at least in normal weight individuals. This relationship is obviously complex with multiple factors involved, but does suggest that ability to increase myocardial ATP delivery is important in determining an individual's performance. The failure to be able to do this in obesity, and in a more pronounced fashion in heart failure, is one potential explanation for the exertional symptoms and more limited functional capacity in the obese cohort as opposed to normal weight. It also suggests that the increased kinetics of the creatine kinase reaction in obesity at rest may come at the cost of lack of exertional response.

The findings of this study are largely consistent with the energetic theory of heart failure – that early changes in, in this case, metabolic flexibility and diastolic function, culminate in systolic dysfunction with depletion of the energetic pool on a progressive continuum. This data also suggests for the first time in humans, that at least in obesity, there is an initial attempt at compensation by increase in creatine kinase kinetics at the cost of ability to augment ATP delivery in response to stress. It is possible that a similar early stage is observed in heart failure at any body weight, prior to the development of systolic dysfunction and symptoms.

5. Conclusions

In this study, I have demonstrated that in addition to lower levels of resting PCr/ATP, in heart failure dobutamine stress leads to further depletion of PCr/ATP. In normal weight heart failure, the increase in creatine kinase reaction rate which occurs in response to stress in normal systolic function is blunted, and therefore overall ATP delivery rates do not increase. In obese heart failure, although baseline creatine kinase rate constant is elevated, maintaining resting ATP delivery, this also fails to augment with stress, leading to an overall fall in ATP delivery. The amount by which creatine kinase activity alters with stress is associated with whole body exercise capacity, suggesting that it may be an important factor in determining exertional symptoms. The implications for our understanding of the pathophysiology of both heart failure and obesity are significant, and raise the question of whether these changes are potentially beneficial, and what the impact of intentional body weight reduction would be.

Chapter Six

The Impact of Weight Loss in Obese Heart Failure

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1. Background

While on the one hand, obesity increases the risk of developing heart failure[2-4], on the other in established disease, there is an obesity paradox in heart failure[14], which demonstrates an apparently improved prognosis in overweight and obese when compared to normal weight, and that weight loss is associated with increased mortality[17-19], even in obese individuals[20]. As a result, current international guidelines either make no recommendation about weight loss, or advise that weight loss in heart failure cannot be endorsed[15, 16]. However, the evidence on which guidelines are based is unable to differentiate intentional weight loss in the contemporary obese, insulin resistant patient, from unintentional loss of muscle mass and cardiac cachexia, a clear marker of poor prognosis[21].

The link between obesity and the development of heart failure is clearly complex and multifactorial; however, a number of factors contribute to increased cardiac work, including increased haemodynamic demand[5], activation of neurohormonal systems, and vascular dysfunction[6]. At the same time, the efficiency of that cardiac work is reduced, due to metabolic derangement[7] leading to depleted energetic stores[25]. It is unsurprising therefore, that obesity is a significant risk factor for the development of heart failure, as well as increasing the severity of the symptoms and functional limitation caused by the disease.

Intentional weight loss in obesity outside the setting of HF results in significant beneficial cardiac remodelling[22, 23], improved metabolism[24, 25, 227], improved prognosis[228] and reduced incidence of heart failure[229]. Whether or not obese patients with HF gain similar benefits is unknown, as is the crucial question of whether intentional weight loss is detrimental in HF.

In previous chapters, I have demonstrated that there are key differences associated with obesity, heart failure, and the combination of the two conditions, in terms of myocardial function and energetics at rest and in the response to stress. Here, I propose to investigate the impact of intentional weight loss on whole body metabolic state, cardiovascular performance, and myocardial high-energy phosphate metabolism in obese heart failure.

2. Methods

For detailed methods see Chapter Two.

In brief, the study was approved by the local ethics board (NRES reference 14/SC/004). 27 obese (BMI>30 kg/m^2) individuals with established dilated cardiomyopathy (EF 25-45%) were recruited from heart failure clinics at a tertiary referral cardiology centre. All signed written consent for the study investigations.

Results were compared to the normal weight heart failure cohort detailed in Chapter Five, and 27 normal weight controls and 45 obese controls with normal systolic function described in Chapter Four.

2.1 Inclusion criteria

Participants were excluded if they had coronary artery disease or severe valvular disease. Additionally, uncontrolled hypertension, atrial fibrillation or diabetes mellitus were exclusion criteria, as was any contraindication to MR scanning.

2.2 Anthropomorphic and biochemical assessment

Height, weight and body composition were measured using digital scales with bio-impedance analysis. Fasting venous blood was taken and total cholesterol, triglycerides, glucose, insulin and BNP were analysed by the Oxford University Hospitals clinical biochemistry laboratory according to standardised protocols. In addition, leptin and adiponectin were analysed using commercially available ELISA kits. Cardiac stroke work was calculated as stroke volume*mean arterial pressure.

2.3 Magnetic resonance imaging

All MRI was obtained using a 3T scanner (Siemens Tim Trio). Abdominal visceral fat, hepatic fat fraction, cardiac structure and systolic function, and myocardial triglyceride content using ^{1}H spectroscopy were acquired as per chapter two.

2.3.1 Phosphorus spectroscopy

^{31}phosphorus spectroscopy was performed on the same 3T system and with the same sequences described in the previous chapter.

2.4 Echocardiography

Echocardiography was performed on a Philips Epiq system, to determine diastolic function; pulse wave velocity was measured at mitral valve inflow and tissue Doppler at lateral and medial mitral valve annulus.

2.5 Cardiopulmonary exercise testing

Volunteers underwent cardiopulmonary exercise testing at each visit, with measurement of maximal capacity on bicycle ergometer, and submaximal capacity on six minute walk test assessment.

2.6 Dietary intervention

All obese participants were assigned a low glycaemic index dietary intervention (1500 kcal/day) for a period of 12 months, before repeat of the study investigations. Phone/email support was available from the study team as instigated by the individual.

2.7 Statistical analysis

Statistical analysis was performed using commercial software (SPSS, Chicago). All data is presented as mean ± standard deviation. Normality of distribution was confirmed before determination of statistical significance, either by Chi-squared tests in the case of categorical data, or student's t tests for continuous data (paired or independent where relevant). Non-parametric data was analysed with the Mann Whitney U test, or Kruskal Wallis test. Pearson's correlation models and simple linear regression were used where relevant. Values of $p<0.05$ were considered as statistically significant.

3. Results

3.1 Success of dietary intervention

Over the study period of 12 months (336 days (IQR 216-432), 8 (30%) individuals from the heart failure cohort did not complete the study (6 undergoing device implantation, and 2 withdrawing consent to follow up). This is higher but statistically similar to the drop-out rate in the normal systolic function control group (17%, p=0.257).

During the study, 12 of the remaining 19 (63%) HF patients lost weight, as defined by any reduction in body weight (mean loss 6±4%, table 6.1). Again the number of successful patients losing weight was statistically similar to the normal systolic function group (82%, p=0.081) although obviously numerically a higher percentage in the control group. The amount of weight lost was higher in the control group (mean -11±5% body weight, p=0.011).

Importantly, as in obese controls with normal systolic function, in HF weight loss was driven by reduction in body fat (by 13±9%, p=0.003) without change in lean muscle mass (p=0.223). This highlights that intentional weight loss is not only achievable in an obese HF population, but additionally is not associated with loss of lean mass.

There were no significant changes in pharmacotherapy in terms of drugs prescribed or doses, brought about by the weight loss intervention.

3.2 Impact of successful weight loss: anthropomorphic and metabolic

In the obese heart failure patient, intentional weight loss was associated with improvements in visceral fat (by 16%, p=0.005, all results in table 6.1), liver fat (by 50%, p=0.026), insulin resistance (HOMA-IR fell by 23%, p=0.029) and leptin levels (by 25%,

p=0.019). While these changes were consistent with what was observed in the obese controls with normal systolic function, there was not a significant change in blood pressure or circulating lipid profile – perhaps because these were addressed pharmacologically to a greater extent at baseline in the HF group.

Table 6.1: The impact of successful weight loss on anthropomorphic and metabolic parameters in obese heart failure compared to individuals with normal systolic function

	Obese controls n=27			Obese heart failure n=12		
	Baseline	**Follow-up**	**p**	**Baseline**	**Follow-up**	**p**
Body mass index (kg/m^2)	36±6	32±5	**<0.001**	35±5	33±5	**0.001**
Body weight (kg)	105±19	94±18	**<0.001**	102±17	97±18	**0.001**
Body fat mass (kg)	44±12	35±12	**<0.001**	42±12	37±12	**0.003**
Fat free mass (kg)	61±14	60±13	**0.001**	58±10	57±10	0.223
Systolic bp (mmHg)	139±24	127±11	**0.014**	133±12	127±13	0.246
Diastolic bp (mmHg)	86±14	81±9	0.167	78±16	78±8	0.921
Metabolic status:						
Abdominal visceral fat (cm^2)	172±79	111±61	**<0.001**	164±100	137±107	**0.005**
Hepatic fat (%)	7.1±6.7	3.1±3.3	**0.001**	3.5±2.6	1.7±1.1	**0.026**
Myocardial triglyceride (%)	2.1±1.3	1.4±0.8	**0.005**	2.1±1.4	1.3±0.5	0.127
Total cholesterol (mmol)	5.5±1.0	4.7±1.0	**0.001**	4.8±1.1	4.8±0.9	1.000
Triglycerides (mmol)	1.7±0.7	1.4±0.8	**0.007**	2.0±1.1	1.4±0.5	0.080
Fasting glucose (mmol)	5.5±0.6	5.0±0.6	**0.001**	5.7±1.6	5.4±1.0	0.658
Fasting insulin (mmol)	111±124	49±25	0.054	94±44	88±65	0.774
HOMA-IR	3.1±1.6	1.6±0.8	**0.003**	3.0±1.2	2.3±1.1	**0.029**
Leptin (ng/ml)	61±40	27±22	**<0.001**	76±51	57±50	**0.019**
BNP (pg/ml)	5.2±2.9	6.5±4.0	0.212	37±39	30±26	0.344

3.3 Impact of successful weight loss: cardiovascular

The results are compared to those achieved by obese individuals with normal systolic function, as presented in Chapter Four; successful weight loss in this control cohort led to reduction in LV EDV (pre 161±39ml vs post 154±38ml, p=0.001), reduction in LV mass (115±33g to 106±26g, p=0.006) and improvement in diastolic function (E/e' fell from 9.0±1.6 to 7.9±1.8, p<0.001).

In obese heart failure, a similar pattern of positive remodelling was seen, with a significant reduction in cavity size (LV EDV 243±71ml to 224±71ml, p=0.049, figure 6.1A) and trend towards regression of LV mass (158±45g vs 148±53g, p=0.053). However, in addition to the geometric remodelling and potentially of more clinical significance, weight loss was associated with a significant improvement in LV systolic function (LVEF +7±5%, p=0.002, figure 6.1C), driven by a greater fall in end-systolic volume (LV ESV 151±64 vs 128±69ml, p=0.008). There was no significant change in LV stroke volume (93±17ml vs 96±22ml, p=0.467), or mean arterial pressure (94±12mmHg vs 93±9mmHg, p=0.977), suggesting that that LV stroke work did not change significantly (p=0.499), in contrast to the normal heart cohort, in which successful weight loss was associated with a 9% fall in stroke work.

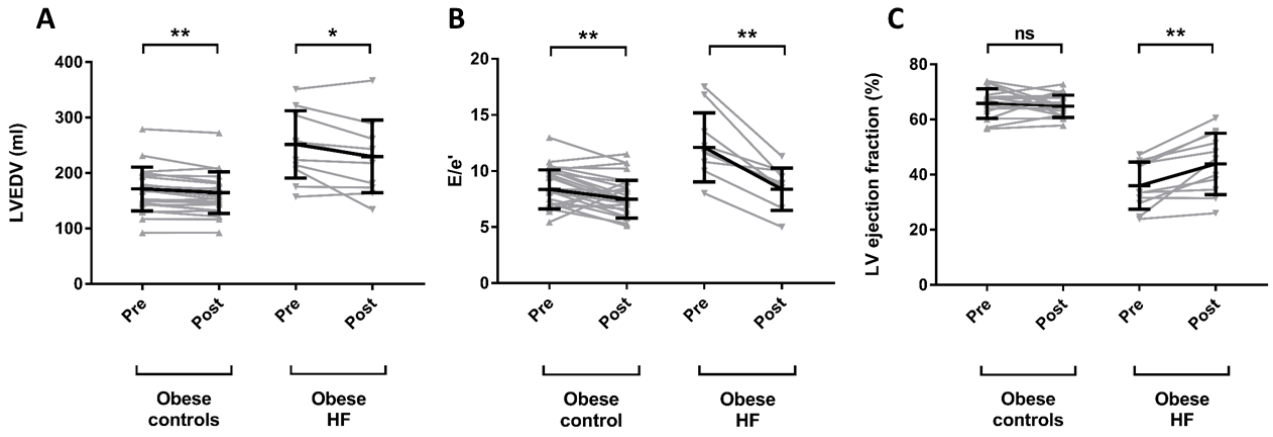


Figure 6.1: The impact of intentional weight loss in obese heart failure compared with controls: (A) left ventricular end-diastolic volume, (B) average E/e' and (C) left ventricular ejection fraction

In obese HFrEF, the change in body weight correlated with change in LVEF (r= -0.590, p=0.013). On linear regression, for every 1kg fall in body weight, LVEF increased by 0.37% (B -0.365, p=0.028). To assess whether the change in contractility was related to change in circulating volume, blood volume was derived from haematocrit and body weight[178]. Using this calculation, there was a significant fall in blood volume with weight loss (a fall by 5±4%, p=0.007); however, this change did not correlate with change in EDV (p=0.406) or EF (p=0.939).

Table 6.2: Impact of successful weight loss on cardiovascular and exercise parameters in heart failure compared with obese controls with normal systolic function

	Obese controls n=27			Obese heart failure n=12		
	Baseline	Follow-up	p	Baseline	Follow-up	p
Left ventricle:						
End-diastolic volume (ml)	160±39	154±38	**0.001**	243±71	224±71	**0.049**
End-systolic volume (ml)	55±16	54±14	0.405	151±64	128±69	**0.008**
Ejection fraction (%)	66±5	65±4	0.126	40±10	47±12	**0.002**
Mass (g)	116±32	105±26	**0.001**	158±45	148±53	0.053
E/e'	8.7±1.7	7.9±1.7	**0.009**	12.6±3.3	8.7±2.0	**0.002**
Right ventricle:						
End-diastolic volume (ml)	151±65	149±70	0.676	163±36	145±45	**0.027**
End-systolic volume (ml)	49±27	50±31	0.522	63±20	55±26	0.205
Ejection fraction (%)	68±5	68±6	0.777	62±7	63±8	0.457
Cardiorespiratory fitness:						
Peak $\dot{V}O_2$ (ml/min/kg)	20±5	20±5	1.000	16±3	17±3	0.529
Exercise time (min)	9±2	9±3	0.607	7±2	7±2	0.329
RER	1.1±0.1	1.1±0.1	0.158	1.1±0.0	1.1±0.0	0.137
Six minute walk test (m)	590±81	611±82	0.074	505±76	532±59	0.057

RV EDV fell (from 163±36ml to 145±45ml, p=0.027), while systolic function remained similar before and after weight loss (RV EF 62±7% to 63±8%, p=0.457). However, there was again a correlation between change in body weight and change in RV EF (r =0.650, p=0.006) across the cohort as a whole.

Interestingly, BNP did not significantly change with weight loss despite the increase in LVEF.

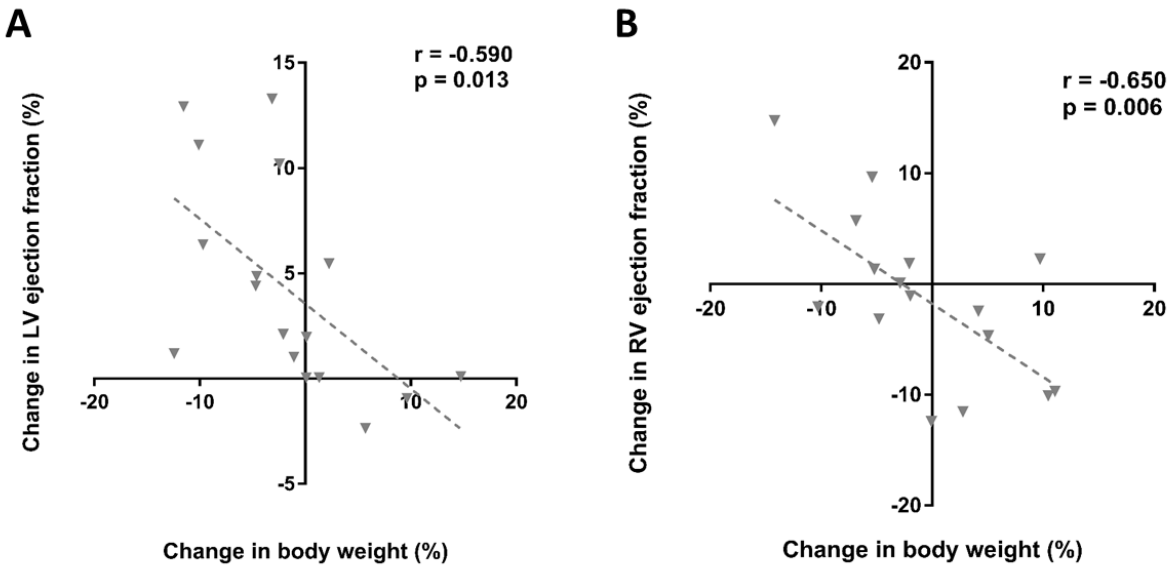


Figure 6.2: The change in LV (A) and RV (B) ejection fraction correlates with change in body weight in obese heart failure

3.4 Effect of weight loss on myocardial energetics

While in the previous chapter, successful weight loss in obese controls was associated with an increase in PCr/ATP, a fall in creatine kinase k_f, and a fall in the overall ATP delivery, although a similar pattern was seen in the heart failure cohort with weight loss (PCr/ATP 1.7±0.3 pre, 1.8±0.5 post, p=0.896; creatine kinase k_f 0.16±0.08s^{-1} pre, 0.12±0.06s^{-1} post, p=0.294; ATP flux (k_f *[PCr]) 1.5±0.8 mmol/g/s pre, 1.1±0.6 mmol/g/s post; p=0.222), these did not reach statistical significance.

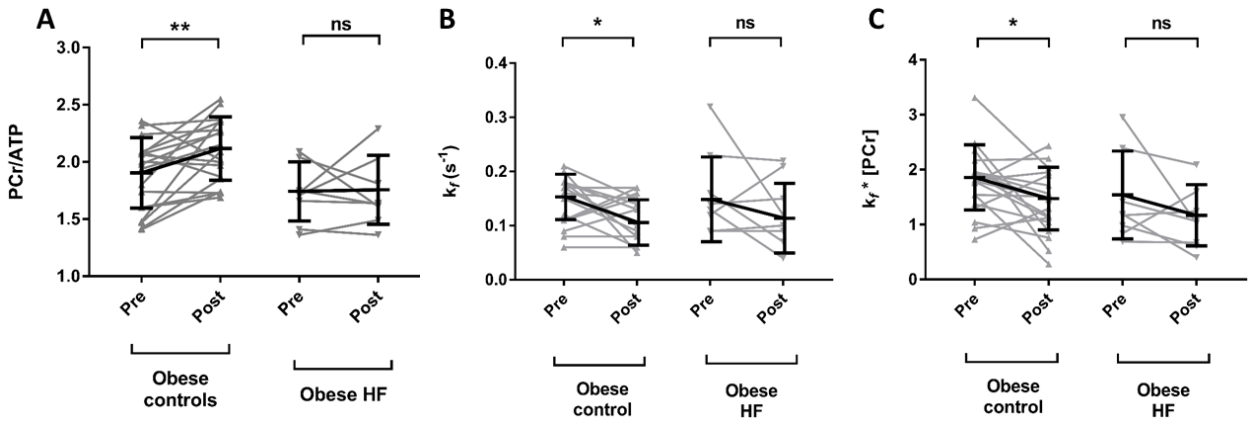


Figure 6.3: The impact of intentional weight loss on myocardial energetics in obese heart failure, compared to controls. (A) PCr/ATP, (B) creatine kinase forward rate constant and (C) ATP delivery

3.5 Weight loss and stress energetics

Only 5 of the heart failure patients who returned for follow-up elected to have and completed dobutamine stress testing. Whilst numbers are low, there is an indication that in these individuals, the fall in PCr/ATP, and overall fall in ATP delivery that was observed with stress in obese HFrEF before dietary weight loss may be reversed. Following weight loss, dobutamine stress led to no significant change in PCr/ATP (rest 1.4±0.1, stress 1.3±0.9, p=0.332), no change in creatine kinase k_f (rest 0.13±0.07 s^{-1}, stress 0.16±0.07s^{-1}), and therefore no overall change in ATP delivery (rest 1.0±0.6 mmol/g/s, stress 1.1±0.4 mmol/g/s, p=0.407). Thus the impact of dobutamine stress on myocardial energetics following successful weight loss is closer to that seen in obese individuals with normal systolic function. Further study of this would be needed with larger numbers to confirm these findings.

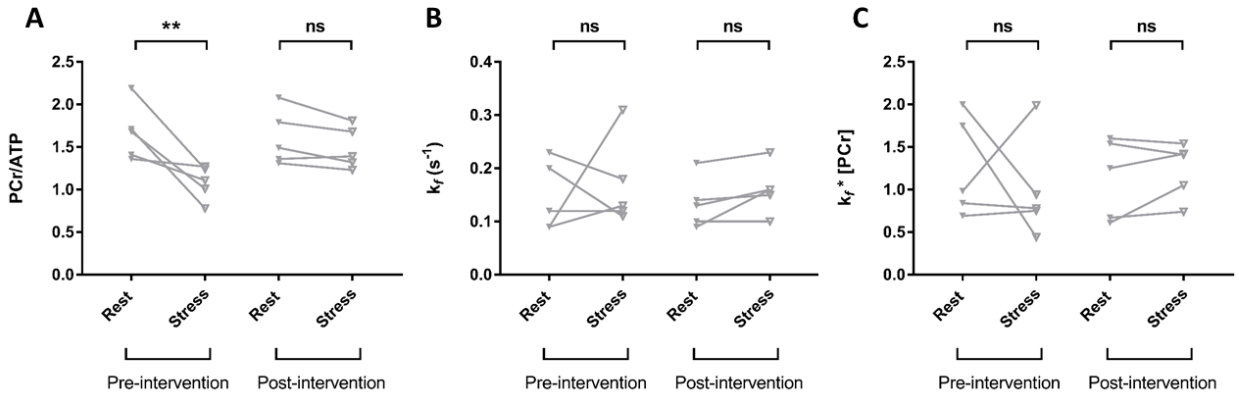


Figure 6.4: The impact of intentional weight loss on the stress response of myocardial energetics in obese heart failure (the stress response is compared in the same 5 individuals pre- and post-weight loss)

3.6 Exercise capacity and weight loss

In contrast to the effect of successful weight loss in obese controls with normal systolic function, where a significant improvement in six minute walk test distance was achieved, in HFrEF there was only a trend towards increase in six minute walk test distance (pre 505±76m, post 532±59m, p=0.057) with this study size. Although there was also no significant improvement in maximal exercise performance seen with weight loss in HFrEF (peak $\dot{V}O_2$ 16±3 ml/kg/min at baseline, 17±3 ml/kg/min post intervention, p=0.529), this was similar to findings in the control group (p>0.9). Overall exercise time and RER were similar pre and post weight loss in both groups.

Thus, while the improvement in exercise capacity observed with weight loss in obese HFrEF was not statistically significant, the magnitude and direction of change was similar to that seen with successful weight loss in the obese group with normal systolic function.

The change in weight correlates with change in six minute walk test distance if both successful and unsuccessful weight loss groups are considered together. However, there is no correlation between the improvement in LV ejection fraction and change in

exercise capacity (for example, for six minute walk test distance p=0.893), or between any changes in measured energetic parameters and change in exercise capacity.

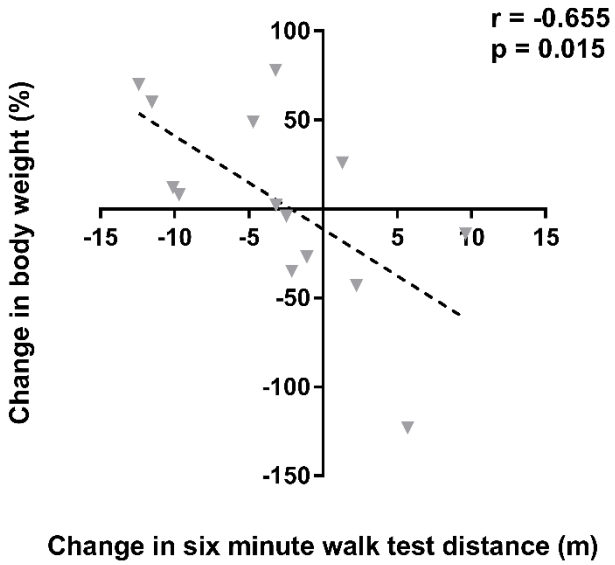


Figure 6.5: Change in body weight correlates with change in six minute walk test distance in obese heart failure as in obese normal systolic function

3.7 Effect of continued obesity

7 obese individuals with heart failure were not successful in losing weight; their weight either remained constant or increased (weight change +4.5±5.3%, p=0.072). In these individuals, there was a significant deterioration in insulin sensitivity (absolute increase in HOMA-IR 6±3, p=0.006 compared to weight loss; table 6.3), and accumulation of myocardial triglyceride (absolute increase in MTGC 1.1±0.7%, p=0.022 compared to weight loss). In contrast to the positive cardiac remodelling seen with weight loss, continued obesity over one year resulted in no improvement in LVEF in patients with HF (absolute change +1±3%, p=0.027 compared to weight loss cohort; table 6.4).

Table 6.3: The impact of continued obesity on anthropomorphic and metabolic parameters in obese heart failure compared to obese controls with normal systolic function

	Obese controls n=8			Obese heart failure n=7		
	Baseline	**Follow-up**	**p**	**Baseline**	**Follow-up**	**p**
Body mass index (kg/m^2)	35±6	36±7	0.104	37±5	38±6	0.064
Body weight (kg)	97±19	101±23	0.113	103±20	109±23	0.073
Body fat mass (kg)	46±12	47±14	0.369	43±10	47±11	0.081
Fat free mass (kg)	53±12	57±15	0.158	66±14	65±14	0.488
Systolic bp (mmHg)	143±17	139±13	0.428	129±16	129±21	0.986
Diastolic bp (mmHg)	85±7	81±12	0.320	75±7	75±8	0.954
Metabolic status:						
Abdominal visceral fat (cm^2)	134±74	120±37	0.582	269±133	291±131	0.100
Hepatic fat (%)	2.3±2.1	3.8±3.5	0.252	6.1±4.2	11.1±4.2	0.053
Myocardial triglyceride (%)	2.1±2.0	2.9±3.3	0.327	1.1±0.7	2.2±0.5	**0.022**
Total cholesterol (mmol)	5.7±1.0	5.5±1.3	0.383	5.1±1.0	4.5±0.9	0.159
Triglycerides (mmol)	1.9±1.2	1.5±0.8	0.406	2.7±1.5	2.3±1.1	0.447
Fasting glucose (mmol)	5.4±0.5	4.8±0.7	0.102	6.9±1.5	8.1±1.4	0.202
Fasting insulin (mmol)	126±94	151±129	0.848	172±86	260±62	**0.042**
HOMA-IR	4.6±3.3	4.4±4.3	0.500	7.3±3.0	13.1±2.3	**0.006**
Leptin (ng/ml)	51±19	39±17	0.083	47±20	30±31	0.192
BNP (pg/ml)	4±2	4±2	0.500	9±3	6±5	0.434

Importantly, in the HF group who did not lose weight, there was a significant fall in peak $\dot{V}O_2$ compared to those who were successful in weight loss (p=0.033). This pattern was also seen in total exercise time, where ongoing obesity led to significant deteriorations compared to weight loss (exercise time fell from 6±2 mins to 4±3 mins, p=0.036 vs weight loss).

Therefore, while no significant improvement in exercise capacity was seen in HF with weight loss, it was maintained, in contrast to deterioration seen in the group with no weight change.

Table 6.4: The impact of continued obesity on cardiac and exercise parameters in obese heart failure compared with obese controls with normal systolic function

	Obese controls n=8			Obese heart failure n=7		
	Baseline	Follow-up	p	Baseline	Follow-up	p
Left ventricle:						
End-diastolic volume (ml)	151±19	145±17	0.226	218±47	215±62	0.107
End-systolic volume (ml)	57±8	56±14	0.859	143±58	118±62	0.190
Ejection fraction (%)	63±2	64±4	0.881	42±10	45±8	0.704
Mass (g)	115±29	110±30	0.398	187±45	177±45	0.618
E/e'	9.9±4.4	10.2±3.2	0.813	8.8±0.4	7.2±1.2	0.144
Right ventricle:						
End-diastolic volume (ml)	150±28	142±16	0.252	155±42	144±55	0.120
End-systolic volume (ml)	50±12	47±8	0.865	69±29	64±28	0.329
Ejection fraction (%)	67±5	67±6	0.884	57±11	55±12	0.363
Cardiopulmonary fitness:						
Peak $\dot{V}O_2$ (ml/kg/min)	16±3	18±2	0.199	17±4	14±4	0.103
Percentage predicted $\dot{V}O_2$	70±38	78±32	0.184	62±20	51±16	0.105
RER	1.1±0.1	1.1±0.1	0.885	1.1±0.0	1.0±0.0	**0.041**
Exercise time (min)	8±2	7±2	0.484	6±2	4±3	**0.036**
Six minute walk test (m)	561±19	553±25	0.459	455±120	417±16	0.309

4. Discussion

The existence of the obesity paradox makes the question of how to manage excess weight in heart failure a significant problem for clinicians worldwide, and one which is currently unresolved[230]. Having demonstrated in previous chapters that obesity in heart failure confers significant metabolic disadvantages in terms of insulin resistance, ectopic lipid deposition and myocardial energy stores as PCr/ATP, it would be expected that weight loss would be beneficial. However, I have also demonstrated that creatine kinase kinetics are elevated in obesity, including in heart failure, whereas low creatine kinase k_f has been linked to limited prognosis and increased heart failure events[96]. Hence, the cardiovascular, whole body and energetic impact of intentional weight loss in obese heart failure is highly relevant.

In this chapter, I have demonstrated that symptomatic obese patients with systolic dysfunction (HFrEF), who intentionally lose weight, can expect to gain significant positive cardiac remodelling in terms of reduced end-diastolic volume. In addition, I have demonstrated that LV systolic function in HFrEF is significantly improved with intentional weight loss. This suggests that intentional fat reduction is not detrimental to contractile function in heart failure. On the contrary, these results show significant improvement in cardiac function and argue that, in obesity, intentional weight loss may be considered as an additional therapeutic strategy in established heart failure.

4.1 Current weight management guidelines in heart failure

All major international guidelines base current recommendations on the results of observational analyses[17-19] of cohorts enrolled in large pharmacological trials, which have

shown two conflicting observations: the apparent mortality benefit of obesity in heart failure (the obesity paradox) and the apparent mortality increase with weight loss in heart failure. As a result, they either make no recommendations, or in the case of the ESC, advise that weight loss cannot be recommended. However, these analyses crucially are unable to differentiate between intentional weight loss and the unintentional cardiac cachexia associated with end-stage disease. In this context, several small studies[26, 27] have suggested that intentional weight loss is linked to exercise and symptom improvements; however, no randomised trials or modern imaging studies exist to guide management[230].

4.2 Intentional weight loss, body composition and risk factor profile

One of the principal concerns regarding intentional weight loss in HF is the potential for this to exacerbate loss of lean muscle mass, which is strongly linked to adverse outcomes[21]. Here I show that weight loss is not only feasible in HF, but importantly, targets visceral, hepatic and subcutaneous fat depots, and does not alter lean muscle mass. In addition, relatively modest amounts of total weight loss (mean 6±4% body weight) result in disproportional reductions in abdominal visceral and hepatic ectopic fat (27-57%), and major improvements in insulin sensitivity. Additional improvements in blood pressure were also observed with weight loss. This indicates that that weight loss in HF is able to further address modifiable risk factors, and indeed that it may be metabolic improvements which drive the cardiovascular changes rather than absolute body weight *per se*.

4.3 Intentional weight loss and cardiac function

Whilst it would seem intuitive that weight loss would be beneficial in HF, to date there has been little evidence to support this. In this study, I have demonstrated here for the

first time, that weight loss in obese HFrEF results in significantly improved systolic function (LVEF by 7%). Importantly LVEF improvement was not only seen across the LVEF spectrum in HFrEF, but also to a level where improved mortality has been shown[231].

As a result, this signposts the potential of weight loss as a widely available, cost effective treatment for HF on top of current medical therapy, which is an exciting proposition. While the exact mechanisms responsible for this improvement are not fully elucidated by this study, several hypotheses can be considered.

4.3.1 Metabolic unloading of the failing ventricle

As already demonstrated particularly in Chapter Three, the myocardium is particularly sensitive to metabolic dysregulation. In both obesity and heart failure, cardiac intracellular metabolism is characterised by inflexibility in substrate selection – the ability in health for the myocyte to switch efficiently between fuel sources in order to maintain maximal work under the prevailing conditions[60] is lost. In other words, both conditions lead to reduced capacity to switch to glucose as a fuel under fed or stressed conditions[69], with associated increased energy costs[83] and depletion of intracellular energy stores[25, 87].

Despite remaining biometrically obese, weight loss in obese HF led to the metabolic profile improving significantly, including both liver fat and insulin resistance. Given the evidence that insulin resistance impairs myocardial performance[232], and improving insulin resistance is linked to improved LV function[233], it is possible that the improvements in insulin resistance seen here with weight loss may play a role in the observed recovery of LV function. In addition, there a numerical fall in myocardial triglyceride content, suggesting that improved peripheral insulin sensitivity may mirror enhanced ability of

the myocardium to combat detrimental excess lipid deposition, and improved metabolic efficiency yielding more myocardial work per unit of energy supplied.

4.3.2 Energetic unloading of the failing ventricle

Intentional weight loss in obesity has been shown to improve both myocardial efficiency[85] and myocardial energetics[25] in the non-failing ventricle.

In contrast to the obese cohort with normal systolic function, in whom weight loss led to replenishment of resting myocardial energetics (PCr/ATP), similar improvements in PCr/ATP were not demonstrated in the HFrEF group despite both successful weight loss, and overall improvement in contractility. This finding suggests that recovery of systolic function is not merely related to increased PCr/ATP stores and that alternative mechanisms are likely to be involved.

Similarly, examination of resting creatine kinase kinetics did not yield any significant changes, in contrast to the obese control group in whom weight loss was associated with a fall in both resting forward rate constant and ATP delivery. However, this may not be that surprising, as overall cardiac stroke work in terms of volumetric change considered in context of afterload, did not change with weight loss, and hence the energetic demand of the myocardium may not be altered.

There is a suggestion of more subtle changes in dynamic energetics, with dobutamine stress leading to maintenance of PCr/ATP and overall ATP delivery, rather than at baseline where stress led to a fall in PCr/ATP and ATP delivery. Although exciting and in line with the findings in a larger cohort in obesity with normal systolic function, this data is drawing heavily on a small number of participants, and needs to be investigated further to be confirmed.

4.3.3 Haemodynamic unloading of the failing ventricle

Traditionally, it was thought that the additional stroke volume demands of obesity, imposed by an increase in total blood volume, caused LV cavity dilatation, eccentric remodelling and contributed to the development of heart failure[234]. As a result, it seems possible that the improved contractility seen here is a consequence of the reduction in body weight, reducing total body blood volume and LV cavity size; indeed, an 8% reduction in LV EDV was seen here, and calculated blood volume fell. This reduction in circulating volume and LV cavity stretch is likely to relieve some of the neurohormonal over-activity seen in HF, and contribute to LV systolic recovery. However, in this study, change in calculated blood volume did not correlate with improvement in LVEF, suggesting that there may be additional factors at play in the heart's response to weight loss, not solely related to haemodynamic unloading.

4.4 Weight loss and improved exercise tolerance

The improvements seen in cardiac function with weight loss in HF were paralleled by improvement of whole body exercise capacity, with increased six-minute walk test distance, correlating with the degree of weight loss. In addition, whereas a fall in exercise capacity was seen with continued obesity, no such deterioration was seen with weight loss. This is likely to reflect a combination of improved cardiac systolic function, alongside the impact of improved metabolic state on skeletal muscle performance, which is not measured here.

5. Conclusions

Obesity and heart failure often coexist, but intentional weight loss remains controversial, and current guidelines provide no definitive advice regarding weight management. Here I have demonstrated that dietary weight loss in obese patients with chronic non-ischaemic heart failure, results in metabolic improvement, a substantial increase in left ventricular systolic function, maintained exercise capacity and possible subtle energetic improvement. Although these findings need to be borne out in larger scale studies, they provide a strong mandate to consider intentional weight loss a potential therapeutic strategy in heart failure.

While the rates of successful voluntary dietary weight loss were lower in the heart failure group than those without concomitant cardiovascular disease, improvements were seen even with relatively modest weight loss. Demonstrating an improvement in an existing disease process is motivating not just for the patient population, but potentially has impact on guidelines and therefore healthcare policy, enabling more investment in behaviour modification programmes which have demonstrated efficacy in multiple studies[235]. Given the frequent relapse which hampers dietary weight loss interventions, bariatric surgery may provide a more invasive, and yet more durable[236] option for the long-term management of obesity-related heart failure.

General Conclusions

Obesity is closely linked to cardiovascular function, and is one of the most common risk factors for developing a wide range of cardiac diseases. However, there is a paradoxical relationship between survival in established disease, and increasing body size. In this thesis, I sought to investigate further the physiology underlying this close and complex interplay, in order to understand more about the development of disease, the potential for obesity to confer beneficial physiological adaptation, and the impact of weight loss on these factors.

Using cutting edge MR techniques, in combination with comprehensive metabolic phenotyping and assessment of cardiopulmonary fitness, I have been able to establish several findings which are entirely novel, and have the potential to change management of obese patients with heart failure.

In Chapter Three, I made use of the modern Very Low Calorie Diet regime to investigate the impact of altered substrate utilisation on cardiovascular function, demonstrating that acutely increasing circulating fatty acids by inducing lipolysis with calorie restriction, is associated with an initial deterioration in both ventricular and vascular function. However, after a period of several weeks, the myocardial steatosis reversed, and improvements in intracellular lipid levels as well as diastolic function were seen. Hence, the power of altering substrate utilisation is demonstrated, as well as proposing a powerful dietary intervention as a treatment for obesity related cardiac dysfunction.

In Chapter Four, I investigated the transfer of ATP from the mitochondrion where it is generated from the substrate in question, to the contractile apparatus of the cell where work is done. This involved novel ^{31}P MRS techniques to enable calculation of the rate of reaction of myocardial creatine kinase, a key enzyme in ATP shuttling, and a factor which has been demonstrated to be sensitively linked to both symptoms and prognosis in heart failure. I demonstrated not only that the rate constant of creatine kinase is increased in obesity, leading to maintained ATP delivery rates in the face of depleted substrate pool, but that the response to stress is blunted in line with impaired functional capacity, and that these factors were all reversible with intentional weight loss. This may provide one mechanism by which adaptation to obesity on a cellular level leads to a protective state, and yet blunted reserve in response to stress indicates potential functional limitation.

In Chapter Five, I sought to establish whether these changes in high-energy phosphate metabolism were also seen in established heart failure, and whether there was any link with cardiac function. I demonstrated that similar results were seen at rest, with depleted PCr/ATP but elevated creatine kinase kinetics, meaning that overall ATP delivery was maintained to a similar level to an obese population with normal systolic function, but significantly greater ATP delivery than normal weight individuals with heart failure. However, with dobutamine stress, PCr/ATP fell significantly, which, with the blunted creatine kinase response, led to an overall fall in ATP delivery. This finding was similar between normal weight and obese heart failure patients, in keeping with reduced exercise capacity in this population.

Finally in Chapter Six, I investigated the impact of intentional weight loss in an obese heart failure cohort, a crucial clinical question which to date is unresolved. I demonstrated that weight loss results in significant improvements in ventricular dimensions and systolic function, which is an exciting proposition for further study and potential translation into clinical practice guidelines. In terms of myocardial energetics, weight loss had more limited impact, with no change in resting PCr/ATP, creatine kinase kinetics or overall ATP delivery. There was a suggestion that there was a restoration of the stress response of creatine kinase, but this needs to be borne out in greater numbers. However, the improvement in systolic function in the absence of energetic replenishment is in itself fascinating, somewhat contradicting the pure energetic theory of heart failure, and raising novel questions about energy demand and supply in the myocardium.

In summary, this body of work adds to our understanding of the pathophysiology linking obesity to cardiac dysfunction, leading to the potential development of novel therapeutic targets, both in pathways manipulating myocardial substrate utilisation, and in the mechanisms by which weight loss is achieved or leads to its associated improvements. I believe treating the burgeoning epidemic of obesity-related cardiac disease will require the deployment of a number of different strategies, but hope that this work demonstrates that it is within our grasp.

References

1. OECD. *Obesity: Past and Projected Future Trends*: OECD Publishing.
2. Hubert HB, Feinleib M, McNamara PM, et al. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation.* 1983;67:968-77.
3. Kenchaiah S, Evans JC, Levy D, et al. Obesity and the risk of heart failure. *N Engl J Med.* 2002;347:305-13.
4. Ho JE, Enserro D, Brouwers FP, et al. Predicting Heart Failure With Preserved and Reduced Ejection Fraction: The International Collaboration on Heart Failure Subtypes. *Circ Heart Fail.* 2016;9.
5. Alexander JK, Dennis EW, Smith WG, et al. Blood volume, cardiac output, and distribution of systemic blood flow in extreme obesity. *Cardiovasc Res Cent Bull.* 1962;1:39-44.
6. Rider OJ, Banerjee R, Rayner JJ, et al. Investigating a Liver Fat: Arterial Stiffening Pathway in Adult and Childhood Obesity. *Arterioscler Thromb Vasc Biol.* 2016;36:198-203.
7. Lopaschuk GD, Folmes CD,Stanley WC. Cardiac energy metabolism in obesity. *Circ Res.* 2007;101:335-47.
8. Rider OJ, Francis JM, Ali MK, et al. Effects of catecholamine stress on diastolic function and myocardial energetics in obesity. *Circulation.* 2012;125:1511-9.
9. Dalos D, Mascherbauer J, Zotter-Tufaro C, et al. Functional Status, Pulmonary Artery Pressure, and Clinical Outcomes in Heart Failure With Preserved Ejection Fraction. *J Am Coll Cardiol.* 2016;68:189-99.
10. Savarese G,Lund LH. Global Public Health Burden of Heart Failure. *Card Fail Rev.* 2017;3:7-11.
11. Conrad N, Judge A, Tran J, et al. Temporal trends and patterns in heart failure incidence: a population-based study of 4 million individuals. *Lancet.* 2018;391:572-580.
12. Eaton CB, Pettinger M, Rossouw J, et al. Risk Factors for Incident Hospitalized Heart Failure With Preserved Versus Reduced Ejection Fraction in a Multiracial Cohort of Postmenopausal Women. *Circ Heart Fail.* 2016;9.
13. Tsao CW, Lyass A, Enserro D, et al. Temporal Trends in the Incidence of and Mortality Associated With Heart Failure With Preserved and Reduced Ejection Fraction. *JACC Heart Fail.* 2018;6:678-685.
14. Oreopoulos A, Padwal R, Kalantar-Zadeh K, et al. Body mass index and mortality in heart failure: a meta-analysis. *Am Heart J.* 2008;156:13-22.
15. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J.* 2016;37:2129-2200.
16. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. *Circulation.* 2013;128:1810-52.

17. Anker SD, Negassa A, Coats AJ, et al. Prognostic importance of weight loss in chronic heart failure and the effect of treatment with angiotensin-converting-enzyme inhibitors: an observational study. *Lancet.* 2003;361:1077-83.
18. Pocock SJ, McMurray JJ, Dobson J, et al. Weight loss and mortality risk in patients with chronic heart failure in the candesartan in heart failure: assessment of reduction in mortality and morbidity (CHARM) programme. *Eur Heart J.* 2008;29:2641-50.
19. Rossignol P, Masson S, Barlera S, et al. Loss in body weight is an independent prognostic factor for mortality in chronic heart failure: insights from the GISSI-HF and Val-HeFT trials. *Eur J Heart Fail.* 2015;17:424-33.
20. Zamora E, Diez-Lopez C, Lupon J, et al. Weight Loss in Obese Patients With Heart Failure. *J Am Heart Assoc.* 2016;5:e002468.
21. Anker SD, Ponikowski P, Varney S, et al. Wasting as independent risk factor for mortality in chronic heart failure. *Lancet.* 1997;349:1050-3.
22. Alpert MA, Terry BE, Lambert CR, et al. Factors influencing left ventricular systolic function in nonhypertensive morbidly obese patients, and effect of weight loss induced by gastroplasty. *Am J Cardiol.* 1993;71:733-7.
23. Haufe S, Utz W, Engeli S, et al. Left ventricular mass and function with reduced-fat or reduced-carbohydrate hypocaloric diets in overweight and obese subjects. *Hypertension.* 2012;59:70-5.
24. Utz W, Engeli S, Haufe S, et al. Moderate dietary weight loss reduces myocardial steatosis in obese and overweight women. *Int J Cardiol.* 2013;167:905-9.
25. Rider OJ, Francis JM, Tyler D, et al. Effects of weight loss on myocardial energetics and diastolic function in obesity. *Int J Cardiovasc Imaging.* 2013;29:1043-50.
26. Mariotti R, Castrogiovanni F, Canale ML, et al. Weight loss and quality of life in chronic heart failure patients. *J Cardiovasc Med (Hagerstown).* 2008;9:576-80.
27. Beck-da-Silva L, Higginson L, Fraser M, et al. Effect of Orlistat in obese patients with heart failure: a pilot study. *Congest Heart Fail.* 2005;11:118-23.
28. Smith HL,Willius FA. Adiposity of the heart. *Arch Intern Med.* 1933;52:911-931.
29. Avelar E, Cloward TV, Walker JM, et al. Left ventricular hypertrophy in severe obesity: interactions among blood pressure, nocturnal hypoxemia, and body mass. *Hypertension.* 2007;49:34-9.
30. Kasper EK, Hruban RH,Baughman KL. Cardiomyopathy of obesity: a clinicopathologic evaluation of 43 obese patients with heart failure. *Am J Cardiol.* 1992;70:921-4.
31. Alpert MA. Obesity cardiomyopathy: pathophysiology and evolution of the clinical syndrome. *Am J Med Sci.* 2001;321:225-36.
32. Alexander JK. Obesity and the heart. *Heart Dis Stroke.* 1993;2:317-21.
33. Ku CS, Lin SL, Wang DJ, et al. Left ventricular filling in young normotensive obese adults. *Am J Cardiol.* 1994;73:613-5.
34. Messerli FH. Cardiopathy of obesity--a not-so-Victorian disease. *N Engl J Med.* 1986;314:378-80.
35. Alpert MA,Hashimi MW. Obesity and the heart. *Am J Med Sci.* 1993;306:117-23.

36. Peterson LR, Waggoner AD, Schechtman KB, et al. Alterations in left ventricular structure and function in young healthy obese women: assessment by echocardiography and tissue Doppler imaging. *J Am Coll Cardiol.* 2004;43:1399-404.
37. Turkbey EB, McClelland RL, Kronmal RA, et al. The impact of obesity on the left ventricle: the Multi-Ethnic Study of Atherosclerosis (MESA). *JACC Cardiovasc Imaging.* 2010;3:266-74.
38. Rider OJ, Petersen SE, Francis JM, et al. Ventricular hypertrophy and cavity dilatation in relation to body mass index in women with uncomplicated obesity. *Heart.* 2011;97:203-8.
39. Rider OJ, Nethononda R, Petersen SE, et al. Concentric left ventricular remodeling and aortic stiffness: a comparison of obesity and hypertension. *Int J Cardiol.* 2013;167:2989-94.
40. Rider OJ, Lewandowski A, Nethononda R, et al. Gender-specific differences in left ventricular remodelling in obesity: insights from cardiovascular magnetic resonance imaging. *Eur Heart J.* 2013;34:292-9.
41. Rider OJ, Lewis AJ, Lewandowski AJ, et al. Obese subjects show sex-specific differences in right ventricular hypertrophy. *Circ Cardiovasc Imaging.* 2015;8.
42. Neeland IJ, Gupta S, Ayers CR, et al. Relation of regional fat distribution to left ventricular structure and function. *Circ Cardiovasc Imaging.* 2013;6:800-7.
43. Gerdts E, Cramariuc D, de Simone G, et al. Impact of left ventricular geometry on prognosis in hypertensive patients with left ventricular hypertrophy (the LIFE study). *Eur J Echocardiogr.* 2008;9:809-15.
44. Krumholz HM, Larson M,Levy D. Prognosis of left ventricular geometric patterns in the Framingham Heart Study. *J Am Coll Cardiol.* 1995;25:879-84.
45. Liao Y, Cooper RS, Mensah GA, et al. Left ventricular hypertrophy has a greater impact on survival in women than in men. *Circulation.* 1995;92:805-10.
46. Lavie CJ, Milani RV, Ventura HO, et al. Disparate effects of left ventricular geometry and obesity on mortality in patients with preserved left ventricular ejection fraction. *Am J Cardiol.* 2007;100:1460-4.
47. Powell BD, Redfield MM, Bybee KA, et al. Association of obesity with left ventricular remodeling and diastolic dysfunction in patients without coronary artery disease. *Am J Cardiol.* 2006;98:116-20.
48. Rider OJ, Francis JM, Ali MK, et al. Determinants of left ventricular mass in obesity; a cardiovascular magnetic resonance study. *J Cardiovasc Magn Reson.* 2009;11:9.
49. Alpert MA, Lavie CJ, Agrawal H, et al. Obesity and heart failure: epidemiology, pathophysiology, clinical manifestations, and management. *Transl Res.* 2014;164:345-56.
50. Tumuklu MM, Etikan I, Kisacik B, et al. Effect of obesity on left ventricular structure and myocardial systolic function: assessment by tissue Doppler imaging and strain/strain rate imaging. *Echocardiography.* 2007;24:802-9.
51. Tanalp AC, Bitigen A, Cevik C, et al. The role of tissue Doppler study in the assessment of left ventricular dysfunction in obesity. *Acta Cardiol.* 2008;63:541-6.
52. Rider OJ, Ajufo E, Ali MK, et al. Myocardial tissue phase mapping reveals impaired myocardial tissue velocities in obesity. *Int J Cardiovasc Imaging.* 2014.

53. Orhan AL, Uslu N, Dayi SU, et al. Effects of isolated obesity on left and right ventricular function: a tissue Doppler and strain rate imaging study. *Echocardiography.* 2010;27:236-43.
54. Abel ED, Litwin SE,Sweeney G. Cardiac remodeling in obesity. *Physiol Rev.* 2008;88:389-419.
55. Appleton CP, Hatle LK,Popp RL. Relation of transmitral flow velocity patterns to left ventricular diastolic function: new insights from a combined hemodynamic and Doppler echocardiographic study. *J Am Coll Cardiol.* 1988;12:426-40.
56. Kossaify A,Nicolas N. Impact of overweight and obesity on left ventricular diastolic function and value of tissue Doppler echocardiography. *Clin Med Insights Cardiol.* 2013;7:43-50.
57. Otto ME, Belohlavek M, Khandheria B, et al. Comparison of right and left ventricular function in obese and nonobese men. *Am J Cardiol.* 2004;93:1569-72.
58. Rider OJ, Francis JM, Ali MK, et al. Beneficial cardiovascular effects of bariatric surgical and dietary weight loss in obesity. *J Am Coll Cardiol.* 2009;54:718-26.
59. Dunlay SM, Roger VL,Redfield MM. Epidemiology of heart failure with preserved ejection fraction. *Nat Rev Cardiol.* 2017;14:591-602.
60. Taegtmeyer H, Golfman L, Sharma S, et al. Linking gene expression to function: metabolic flexibility in the normal and diseased heart. *Ann N Y Acad Sci.* 2004;1015:202-13.
61. Uphues I, Kolter T, Goud B, et al. Failure of insulin-regulated recruitment of the glucose transporter GLUT4 in cardiac muscle of obese Zucker rats is associated with alterations of small-molecular-mass GTP-binding proteins. *Biochem J.* 1995;311 (Pt 1):161-6.
62. Berger J,Moller DE. The mechanisms of action of PPARs. *Annu Rev Med.* 2002;53:409-35.
63. Motojima K, Passilly P, Peters JM, et al. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. *J Biol Chem.* 1998;273:16710-4.
64. Van Bilsen M, de Vries JE,Van der Vusse GJ. Long-term effects of fatty acids on cell viability and gene expression of neonatal cardiac myocytes. *Prostaglandins Leukot Essent Fatty Acids.* 1997;57:39-45.
65. van der Lee KA, Vork MM, De Vries JE, et al. Long-chain fatty acid-induced changes in gene expression in neonatal cardiac myocytes. *J Lipid Res.* 2000;41:41-7.
66. Distel RJ, Robinson GS,Spiegelman BM. Fatty acid regulation of gene expression. Transcriptional and post-transcriptional mechanisms. *J Biol Chem.* 1992;267:5937-41.
67. van der Vusse GJ, Glatz JF, Stam HC, et al. Fatty acid homeostasis in the normoxic and ischemic heart. *Physiol Rev.* 1992;72:881-940.
68. Coort SL, Hasselbaink DM, Koonen DP, et al. Enhanced sarcolemmal FAT/CD36 content and triacylglycerol storage in cardiac myocytes from obese zucker rats. *Diabetes.* 2004;53:1655-63.
69. Neubauer S. The failing heart--an engine out of fuel. *N Engl J Med.* 2007;356:1140-51.

70. Le Page LM, Rider OJ, Lewis AJ, et al. Increasing Pyruvate Dehydrogenase Flux as a Treatment for Diabetic Cardiomyopathy: A Combined 13C Hyperpolarized Magnetic Resonance and Echocardiography Study. *Diabetes*. 2015.
71. Djouadi F, Brandt JM, Weinheimer CJ, et al. The role of the peroxisome proliferator-activated receptor alpha (PPAR alpha) in the control of cardiac lipid metabolism. *Prostaglandins Leukot Essent Fatty Acids*. 1999;60:339-43.
72. Zhou YT, Grayburn P, Karim A, et al. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A*. 2000;97:1784-9.
73. Unger RH. Lipotoxic diseases. *Annu Rev Med*. 2002;53:319-36.
74. Vincent HK, Powers SK, Stewart DJ, et al. Obesity is associated with increased myocardial oxidative stress. *Int J Obes Relat Metab Disord*. 1999;23:67-74.
75. Hue L,Taegtmeyer H. The Randle cycle revisited: a new head for an old hat. *Am J Physiol Endocrinol Metab*. 2009;297:E578-91.
76. Young ME, Guthrie PH, Razeghi P, et al. Impaired long-chain fatty acid oxidation and contractile dysfunction in the obese Zucker rat heart. *Diabetes*. 2002;51:2587-95.
77. Hardie DG, Corton J, Ching YP, et al. Regulation of lipid metabolism by the AMP-activated protein kinase. *Biochem Soc Trans*. 1997;25:1229-31.
78. Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology*. 2003;144:5179-83.
79. Martin TL, Alquier T, Asakura K, et al. Diet-induced obesity alters AMP kinase activity in hypothalamus and skeletal muscle. *J Biol Chem*. 2006;281:18933-41.
80. Veech RL. The determination of the redox states and phosphorylation potential in living tissues and their relationship to metabolic control of disease phenotypes. *Biochem Mol Biol Educ*. 2006;34:168-79.
81. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70:309-19.
82. Cole MA, Murray AJ, Cochlin LE, et al. A high fat diet increases mitochondrial fatty acid oxidation and uncoupling to decrease efficiency in rat heart. *Basic Res Cardiol*. 2011;106:447-57.
83. Simonsen S,Kjekshus JK. The effect of free fatty acids on myocardial oxygen consumption during atrial pacing and catecholamine infusion in man. *Circulation*. 1978;58:484-91.
84. Peterson LR, Herrero P, Schechtman KB, et al. Effect of obesity and insulin resistance on myocardial substrate metabolism and efficiency in young women. *Circulation*. 2004;109:2191-6.
85. Lin CH, Kurup S, Herrero P, et al. Myocardial oxygen consumption change predicts left ventricular relaxation improvement in obese humans after weight loss. *Obesity (Silver Spring)*. 2011;19:1804-12.
86. Bessman SP,Geiger PJ. Transport of energy in muscle: the phosphorylcreatine shuttle. *Science*. 1981;211:448-52.
87. Neubauer S, Krahe T, Schindler R, et al. P-31 Magnetic-Resonance Spectroscopy in Dilated Cardiomyopathy and Coronary-Artery Disease - Altered Cardiac High-Energy Phosphate-Metabolism in Heart-Failure. *Circulation*. 1992;86:1810-1818.

88. Neubauer S, Horn M, Cramer M, et al. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation.* 1997;96:2190-6.
89. Neubauer S, Horn M, Pabst T, et al. Contributions of 31P-magnetic resonance spectroscopy to the understanding of dilated heart muscle disease. *Eur Heart J.* 1995;16 Suppl O:115-8.
90. Dass S, Cochlin LE, Suttie JJ, et al. Exacerbation of cardiac energetic impairment during exercise in hypertrophic cardiomyopathy: a potential mechanism for diastolic dysfunction. *Eur Heart J.* 2015;36:1547-54.
91. Levelt E, Rodgers CT, Clarke WT, et al. Cardiac energetics, oxygenation, and perfusion during increased workload in patients with type 2 diabetes mellitus. *Eur Heart J.* 2015;65:44-52.
92. Wijesurendra RS, Liu A, Eichhorn C, et al. Lone Atrial Fibrillation Is Associated With Impaired Left Ventricular Energetics That Persists Despite Successful Catheter Ablation. *Circulation.* 2016;134:1068-1081.
93. Weiss RG, Gerstenblith G,Bottomley PA. ATP flux through creatine kinase in the normal, stressed, and failing human heart. *Proc Natl Acad Sci U S A.* 2005;102:808-13.
94. Bottomley PA, Wu KC, Gerstenblith G, et al. Reduced myocardial creatine kinase flux in human myocardial infarction: an in vivo phosphorus magnetic resonance spectroscopy study. *Circulation.* 2009;119:1918-24.
95. Smith CS, Bottomley PA, Schulman SP, et al. Altered creatine kinase adenosine triphosphate kinetics in failing hypertrophied human myocardium. *Circulation.* 2006;114:1151-8.
96. Bottomley PA, Panjrath GS, Lai S, et al. Metabolic rates of ATP transfer through creatine kinase (CK Flux) predict clinical heart failure events and death. *Sci Transl Med.* 2013;5:215re3.
97. Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994;372:425-32.
98. Tartaglia LA, Dembski M, Weng X, et al. Identification and expression cloning of a leptin receptor, OB-R. *Cell.* 1995;83:1263-71.
99. Lee Y, Wang MY, Kakuma T, et al. Liporegulation in diet-induced obesity. The antisteatotic role of hyperleptinemia. *J Biol Chem.* 2001;276:5629-35.
100. Chiu HC, Kovacs A, Ford DA, et al. A novel mouse model of lipotoxic cardiomyopathy. *J Clin Invest.* 2001;107:813-22.
101. Lee Y, Naseem RH, Duplomb L, et al. Hyperleptinemia prevents lipotoxic cardiomyopathy in acyl CoA synthase transgenic mice. *Proc Natl Acad Sci U S A.* 2004;101:13624-9.
102. Martin SS, Qasim A,Reilly MP. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. *J Am Coll Cardiol.* 2008;52:1201-10.
103. Chen K, Li F, Li J, et al. Induction of leptin resistance through direct interaction of C-reactive protein with leptin. *Nat Med.* 2006;12:425-32.
104. Unger RH. Hyperleptinemia: protecting the heart from lipid overload. *Hypertension.* 2005;45:1031-4.

105. Madani S, De Girolamo S, Munoz DM, et al. Direct effects of leptin on size and extracellular matrix components of human pediatric ventricular myocytes. *Cardiovasc Res.* 2006;69:716-25.
106. McGaffin KR, Moravec CS,McTiernan CF. Leptin signaling in the failing and mechanically unloaded human heart. *Circ Heart Fail.* 2009;2:676-83.
107. Wannamethee SG, Shaper AG, Whincup PH, et al. The obesity paradox in men with coronary heart disease and heart failure: the role of muscle mass and leptin. *Int J Cardiol.* 2014;171:49-55.
108. Skurk C, Wittchen F, Suckau L, et al. Description of a local cardiac adiponectin system and its deregulation in dilated cardiomyopathy. *Eur Heart J.* 2008;29:1168-80.
109. Hotta K, Funahashi T, Bodkin NL, et al. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes.* 2001;50:1126-33.
110. Chandran M, Phillips SA, Ciaraldi T, et al. Adiponectin: more than just another fat cell hormone? *Diabetes Care.* 2003;26:2442-50.
111. Palanivel R, Fang X, Park M, et al. Globular and full-length forms of adiponectin mediate specific changes in glucose and fatty acid uptake and metabolism in cardiomyocytes. *Cardiovasc Res.* 2007;75:148-57.
112. Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med.* 2001;7:941-6.
113. Shibata R, Ouchi N, Ito M, et al. Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat Med.* 2004;10:1384-9.
114. McManus DD, Lyass A, Ingelsson E, et al. Relations of circulating resistin and adiponectin and cardiac structure and function: the Framingham Offspring Study. *Obesity (Silver Spring).* 2012;20:1882-6.
115. Kistorp C, Faber J, Galatius S, et al. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation.* 2005;112:1756-1762.
116. Antonopoulos AS, Margaritis M, Verheule S, et al. Mutual Regulation of Epicardial Adipose Tissue and Myocardial Redox State by PPAR-gamma/Adiponectin Signalling. *Circ Res.* 2016;118:842-55.
117. Madamanchi C, Alhosaini H, Sumida A, et al. Obesity and natriuretic peptides, BNP and NT-proBNP: Mechanisms and diagnostic implications for heart failure. *Int J Cardiol.* 2014;176:611-617.
118. Clerico A, Giannoni A, Vittorini S, et al. The paradox of low BNP levels in obesity. *Heart Fail Rev.* 2012;17:81-96.
119. Mukoyama M, Nakao K, Hosoda K, et al. Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. *J Clin Invest.* 1991;87:1402-12.
120. Frankenstein L, Zugck C, Nelles M, et al. The obesity paradox in stable chronic heart failure does not persist after matching for indicators of disease severity and confounders. *Eur J Heart Fail.* 2009;11:1189-94.
121. Wang TJ, Larson MG, Levy D, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med.* 2004;350:655-63.

122. St Peter JV, Hartley GG, Murakami MM, et al. B-type natriuretic peptide (BNP) and N-terminal pro-BNP in obese patients without heart failure: relationship to body mass index and gastric bypass surgery. *Clin Chem.* 2006;52:680-5.
123. Chainani-Wu N, Weidner G, Purnell DM, et al. Relation of B-type natriuretic peptide levels to body mass index after comprehensive lifestyle changes. *Am J Cardiol.* 2010;105:1570-6.
124. Kitzman DW,Shah SJ. The HFpEF Obesity Phenotype: The Elephant in the Room. *J Am Coll Cardiol.* 2016;68:200-3.
125. Troisi RJ, Weiss ST, Parker DR, et al. Relation of obesity and diet to sympathetic nervous system activity. *Hypertension.* 1991;17:669-77.
126. Grassi G, Seravalle G, Cattaneo BM, et al. Sympathetic activation in obese normotensive subjects. *Hypertension.* 1995;25:560-3.
127. Landsberg L. Diet, obesity and hypertension: an hypothesis involving insulin, the sympathetic nervous system, and adaptive thermogenesis. *Q J Med.* 1986;61:1081-90.
128. Guarino D, Nannipieri M, Iervasi G, et al. The Role of the Autonomic Nervous System in the Pathophysiology of Obesity. *Front Physiol.* 2017;8:665.
129. Mahu I,Domingos AI. The sympathetic neuro-adipose connection and the control of body weight. *Exp Cell Res.* 2017;360:27-30.
130. Zeng W, Pirzgalska RM, Pereira MM, et al. Sympathetic neuro-adipose connections mediate leptin-driven lipolysis. *Cell.* 2015;163:84-94.
131. Mark AL, Correia ML, Rahmouni K, et al. Selective leptin resistance: a new concept in leptin physiology with cardiovascular implications. *J Hypertens.* 2002;20:1245-50.
132. Alvarez GE, Ballard TP, Beske SD, et al. Subcutaneous obesity is not associated with sympathetic neural activation. *Am J Physiol Heart Circ Physiol.* 2004;287:H414-8.
133. Kalantar-Zadeh K, Block G, Humphreys MH, et al. Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. *Kidney Int.* 2003;63:793-808.
134. Kremers HM, Nicola PJ, Crowson CS, et al. Prognostic importance of low body mass index in relation to cardiovascular mortality in rheumatoid arthritis. *Arthritis Rheum.* 2004;50:3450-7.
135. Marti S, Munoz X, Rios J, et al. Body weight and comorbidity predict mortality in COPD patients treated with oxygen therapy. *Eur Respir J.* 2006;27:689-96.
136. Hoit BD, Gilpin EA, Maisel AA, et al. Influence of obesity on morbidity and mortality after acute myocardial infarction. *Am Heart J.* 1987;114:1334-41.
137. Niedziela J, Hudzik B, Niedziela N, et al. The obesity paradox in acute coronary syndrome: a meta-analysis. *Eur J Epidemiol.* 2014;29:801-12.
138. Clark AL, Chyu J,Horwich TB. The obesity paradox in men versus women with systolic heart failure. *Am J Cardiol.* 2012;110:77-82.
139. Patel DA, Lavie CJ, Artham SM, et al. Effects of left ventricular geometry and obesity on mortality in women with normal ejection fraction. *Am J Cardiol.* 2014;113:877-80.
140. Clark AL, Fonarow GC,Horwich TB. Obesity and the obesity paradox in heart failure. *Prog Cardiovasc Dis.* 2014;56:409-14.
141. Fonarow GC, Srikanthan P, Costanzo MR, et al. An obesity paradox in acute heart failure: analysis of body mass index and inhospital mortality for 108,927 patients in

the Acute Decompensated Heart Failure National Registry. *Am Heart J.* 2007;153:74-81.
142. Shah R, Gayat E, Januzzi JL, Jr., et al. Body mass index and mortality in acutely decompensated heart failure across the world: a global obesity paradox. *J Am Coll Cardiol.* 2014;63:778-85.
143. Komukai K, Minai K, Arase S, et al. Impact of body mass index on clinical outcome in patients hospitalized with congestive heart failure. *Circ J.* 2012;76:145-51.
144. Wang J, Yang YM, Zhu J, et al. Obesity paradox in patients with atrial fibrillation and heart failure. *Int J Cardiol.* 2014;176:1356-8.
145. Casas-Vara A, Santolaria F, Fernandez-Bereciartua A, et al. The obesity paradox in elderly patients with heart failure: analysis of nutritional status. *Nutrition.* 2012;28:616-22.
146. Kapoor JR,Heidenreich PA. Obesity and survival in patients with heart failure and preserved systolic function: a U-shaped relationship. *Am Heart J.* 2010;159:75-80.
147. Padwal R, McAlister FA, McMurray JJ, et al. The obesity paradox in heart failure patients with preserved versus reduced ejection fraction: a meta-analysis of individual patient data. *Int J Obes (Lond).* 2014;38:1110-4.
148. Nagarajan V, Cauthen CA, Starling RC, et al. Prognosis of morbid obesity patients with advanced heart failure. *Congest Heart Fail.* 2013;19:160-4.
149. Zamora E, Lupon J, de Antonio M, et al. The obesity paradox in heart failure: is etiology a key factor? *Int J Cardiol.* 2013;166:601-5.
150. Arena R, Myers J, Abella J, et al. Influence of etiology of heart failure on the obesity paradox. *Am J Cardiol.* 2009;104:1116-21.
151. Adamopoulos C, Meyer P, Desai RV, et al. Absence of obesity paradox in patients with chronic heart failure and diabetes mellitus: a propensity-matched study. *Eur J Heart Fail.* 2011;13:200-6.
152. Pinho E, Lourenco P, Silva S, et al. Higher BMI in heart failure patients is associated with longer survival only in the absence of diabetes. *J Cardiovasc Med (Hagerstown).* 2014.
153. Guglin M, Baxi K,Schabath M. Anatomy of the obesity paradox in heart failure. *Heart Fail Rev.* 2014;19:621-35.
154. Oreopoulos A, Ezekowitz JA, McAlister FA, et al. Association between direct measures of body composition and prognostic factors in chronic heart failure. *Mayo Clin Proc.* 2010;85:609-17.
155. Lavie CJ, Osman AF, Milani RV, et al. Body composition and prognosis in chronic systolic heart failure: the obesity paradox. *Am J Cardiol.* 2003;91:891-4.
156. Cicoira M, Doehner W, Jankowska E, et al. Body composition and prognosis in 511 chronic heart failure patients: results from three European centers. *European Journal of Heart Failure Supplements.* 2004;3:69.
157. Futter JE, Cleland JG,Clark AL. Body mass indices and outcome in patients with chronic heart failure. *Eur J Heart Fail.* 2011;13:207-13.
158. Oreopoulos A, Kalantar-Zadeh K, McAlister FA, et al. Comparison of direct body composition assessment methods in patients with chronic heart failure. *J Card Fail.* 2010;16:867-72.
159. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999;257:79-83.

160. Yang WS, Lee WJ, Funahashi T, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab.* 2001;86:3815-9.
161. Lavie CJ, Cahalin LP, Chase P, et al. Impact of cardiorespiratory fitness on the obesity paradox in patients with heart failure. *Mayo Clin Proc.* 2013;88:251-8.
162. Zafrir B, Salman N,Amir O. Joint impact of body mass index and physical capacity on mortality in patients with systolic heart failure. *Am J Cardiol.* 2014;113:1217-21.
163. Chase P, Arena R, Myers J, et al. Relation of the prognostic value of ventilatory efficiency to body mass index in patients with heart failure. *Am J Cardiol.* 2008;101:348-52.
164. Anker SD, Swan JW, Volterrani M, et al. The influence of muscle mass, strength, fatigability and blood flow on exercise capacity in cachectic and non-cachectic patients with chronic heart failure. *Eur Heart J.* 1997;18:259-69.
165. Zavin A, Daniels K, Arena R, et al. Adiposity facilitates increased strength capacity in heart failure patients with reduced ejection fraction. *Int J Cardiol.* 2013;167:2468-71.
166. Lavie CJ, Alpert MA, Arena R, et al. Impact of obesity and the obesity paradox on prevalence and prognosis in heart failure. *JACC Heart Fail.* 2013;1:93-102.
167. Lavie CJ, De Schutter A, Alpert MA, et al. Obesity paradox, cachexia, frailty, and heart failure. *Heart Fail Clin.* 2014;10:319-26.
168. McAuley PA, Artero EG, Sui X, et al. The obesity paradox, cardiorespiratory fitness, and coronary heart disease. *Mayo Clin Proc.* 2012;87:443-51.
169. McAuley PA,Beavers KM. Contribution of cardiorespiratory fitness to the obesity paradox. *Prog Cardiovasc Dis.* 2014;56:434-40.
170. Grapsa J, Tan TC, Paschou SA, et al. The effect of bariatric surgery on echocardiographic indices: a review of the literature. *Eur J Clin Invest.* 2013;43:1224-30.
171. Di Bello V, Santini F, Di Cori A, et al. Effects of bariatric surgery on early myocardial alterations in adult severely obese subjects. *Cardiology.* 2008;109:241-8.
172. Isbell JM, Tamboli RA, Hansen EN, et al. The importance of caloric restriction in the early improvements in insulin sensitivity after Roux-en-Y gastric bypass surgery. *Diabetes Care.* 2010;33:1438-42.
173. Dirksen C, Jorgensen NB, Bojsen-Moller KN, et al. Mechanisms of improved glycaemic control after Roux-en-Y gastric bypass. *Diabetologia.* 2012;55:1890-901.
174. Woelnerhanssen B, Peterli R, Steinert RE, et al. Effects of postbariatric surgery weight loss on adipokines and metabolic parameters: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy--a prospective randomized trial. *Surg Obes Relat Dis.* 2011;7:561-8.
175. Vest AR, Patel P, Schauer PR, et al. Clinical and Echocardiographic Outcomes After Bariatric Surgery in Obese Patients With Left Ventricular Systolic Dysfunction. *Circ Heart Fail.* 2016;9:e002260.
176. Gill RS, Karmali S, Nagandran J, et al. Combined Ventricular Assist Device Placement With Adjustable Gastric Band (VAD-BAND): A Promising New Technique for Morbidly Obese Patients Awaiting Potential Cardiac Transplantation. *J Clin Med Res.* 2012;4:127-9.

177. Ramani GV, McCloskey C, Ramanathan RC, et al. Safety and efficacy of bariatric surgery in morbidly obese patients with severe systolic heart failure. *Clin Cardiol.* 2008;31:516-20.
178. Ling HZ, Flint J, Damgaard M, et al. Calculated plasma volume status and prognosis in chronic heart failure. *Eur J Heart Fail.* 2015;17:35-43.
179. Abate N, Garg A, Coleman R, et al. Prediction of total subcutaneous abdominal, intraperitoneal, and retroperitoneal adipose tissue masses in men by a single axial magnetic resonance imaging slice. *Am J Clin Nutr.* 1997;65:403-8.
180. Reeder SB, Pineda AR, Wen Z, et al. Iterative decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL): application with fast spin-echo imaging. *Magn Reson Med.* 2005;54:636-44.
181. Rial B, Robson MD, Neubauer S, et al. Rapid quantification of myocardial lipid content in humans using single breath-hold 1H MRS at 3 Tesla. *Magnetic Resonance in Medicine.* 66:619-624.
182. Rayner JJ, Banerjee R, Holloway CJ, et al. The relative contribution of metabolic and structural abnormalities to diastolic dysfunction in obesity. *Int J Obes (Lond).* 2017.
183. Schar M, El-Sharkawy AM, Weiss RG, et al. Triple repetition time saturation transfer (TRiST) 31P spectroscopy for measuring human creatine kinase reaction kinetics. *Magn Reson Med.* 2010;63:1493-501.
184. Schar M, Gabr RE, El-Sharkawy AM, et al. Two repetition time saturation transfer (TwiST) with spill-over correction to measure creatine kinase reaction rates in human hearts. *J Cardiovasc Magn Reson.* 2015;17:70.
185. Clarke WT, Peterzan MA, Rayner JJ, et al. Localized rest and stress human cardiac creatine kinase reaction kinetics at 3 T. *NMR Biomed.* 2019:e4085.
186. Okin PM, Lauer MS,Kligfield P. Chronotropic response to exercise. Improved performance of ST-segment depression criteria after adjustment for heart rate reserve. *Circulation.* 1996;94:3226-31.
187. Holland AE, Spruit MA, Troosters T, et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. *Eur Respir J.* 2014;44:1428-46.
188. Jenkins DJ, Wolever TM, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr.* 1981;34:362-6.
189. Brand-Miller JC, Petocz P,Colagiuri S. Meta-analysis of low-glycemic index diets in the management of diabetes: response to Franz. *Diabetes Care.* 2003;26:3363-4; author reply 3364-5.
190. Schwingshackl L,Hoffmann G. Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. *Nutr Metab Cardiovasc Dis.* 2013;23:699-706.
191. Kelly S, Frost G, Whittaker V, et al. Low glycaemic index diets for coronary heart disease. *Cochrane Database Syst Rev.* 2004:CD004467.
192. Gallop R. *The Gi diet : the Glycemic Index.* London: Virgin Books; 2003.
193. Lean ME, Leslie WS, Barnes AC, et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *Lancet.* 2017.
194. Rider OJ, Cox P, Tyler D, et al. Myocardial substrate metabolism in obesity. *Int J Obes (Lond).* 2013;37:972-9.

195. Minokoshi Y, Kim YB, Peroni OD, et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature.* 2002;415:339-43.
196. Rider OJ, Holloway CJ, Emmanuel Y, et al. Increasing plasma free fatty acids in healthy subjects induces aortic distensibility changes seen in obesity. *Circ Cardiovasc Imaging.* 2012;5:367-75.
197. Hexeberg S, Hessevik I,Hexeberg E. Intravenous lipid infusion results in myocardial lipid droplet accumulation combined with reduced myocardial performance in heparinized rabbits. *Acta Physiol Scand.* 1995;153:159-68.
198. Ge F, Hu C, Hyodo E, et al. Cardiomyocyte triglyceride accumulation and reduced ventricular function in mice with obesity reflect increased long chain Fatty Acid uptake and de novo Fatty Acid synthesis. *J Obes.* 2012;2012:205648.
199. Jonker JT, Djaberi R, van Schinkel LD, et al. Very-low-calorie diet increases myocardial triglyceride content and decreases diastolic left ventricular function in type 2 diabetes with cardiac complications. *Diabetes Care.* 2014;37:e1-2.
200. Hammer S, van der Meer RW, Lamb HJ, et al. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab.* 2008;295:E714-8.
201. Sours HE, Frattali VP, Brand CD, et al. Sudden death associated with very low calorie weight reduction regimens. *Am J Clin Nutr.* 1981;34:453-61.
202. Kerr M, Dodd MS,Heather LC. The 'Goldilocks zone' of fatty acid metabolism; to ensure that the relationship with cardiac function is just right. *Clin Sci (Lond).* 2017;131:2079-2094.
203. Banerjee R, Rial B, Holloway CJ, et al. Evidence of a Direct Effect of Myocardial Steatosis on LV Hypertrophy and Diastolic Dysfunction in Adult and Adolescent Obesity. *JACC Cardiovasc Imaging.* 2015;8:1468-1470.
204. Mehra MR, Uber PA, Park MH, et al. Obesity and suppressed B-type natriuretic peptide levels in heart failure. *J Am Coll Cardiol.* 2004;43:1590-5.
205. Carvajal K, Balderas-Villalobos J, Bello-Sanchez MD, et al. Ca(2+) mishandling and cardiac dysfunction in obesity and insulin resistance: role of oxidative stress. *Cell Calcium.* 2014;56:408-15.
206. Mizuno Y, Harada E, Nakagawa H, et al. The diabetic heart utilizes ketone bodies as an energy source. *Metabolism.* 2017;77:65-72.
207. Yu JG, Javorschi S, Hevener AL, et al. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes.* 2002;51:2968-74.
208. Kappes A,Loffler G. Influences of ionomycin, dibutyryl-cycloAMP and tumour necrosis factor-alpha on intracellular amount and secretion of apM1 in differentiating primary human preadipocytes. *Horm Metab Res.* 2000;32:548-54.
209. Chinen I, Shimabukuro M, Yamakawa K, et al. Vascular lipotoxicity: endothelial dysfunction via fatty-acid-induced reactive oxygen species overproduction in obese Zucker diabetic fatty rats. *Endocrinology.* 2007;148:160-5.
210. Davda RK, Stepniakowski KT, Lu G, et al. Oleic acid inhibits endothelial nitric oxide synthase by a protein kinase C-independent mechanism. *Hypertension.* 1995;26:764–70.
211. Steinberg HO, Paradisi G, Hook G, et al. Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. *Diabetes.* 2000;49:1231-8.

212. Steinberg HO, Tarshoby M, Monestel R, et al. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. *J Clin Invest.* 1997;100:1230-9.
213. Bashir A, Coggan AR,Gropler RJ. In vivo creatine kinase reaction kinetics at rest and stress in type II diabetic rat heart. *Physiol Rep.* 2015;3.
214. Hittel DS, Hathout Y, Hoffman EP, et al. Proteome analysis of skeletal muscle from obese and morbidly obese women. *Diabetes.* 2005;54:1283-8.
215. Tome MA, Botana MA, Cadarso-Suarez C, et al. Prevalence of metabolic syndrome in Galicia (NW Spain) on four alternative definitions and association with insulin resistance. *J Endocrinol Invest.* 2009;32:505-11.
216. Abraham MR, Bottomley PA, Dimaano VL, et al. Creatine kinase adenosine triphosphate and phosphocreatine energy supply in a single kindred of patients with hypertrophic cardiomyopathy. *Am J Cardiol.* 2013;112:861-6.
217. Nascimben L, Ingwall JS, Pauletto P, et al. Creatine kinase system in failing and nonfailing human myocardium. *Circulation.* 1996;94:1894-901.
218. Park SJ, Zhang J, Ye Y, et al. Myocardial creatine kinase expression after left ventricular assist device support. *J Am Coll Cardiol.* 2002;39:1773-9.
219. Lin G, Liu Y,MacLeod KM. Regulation of muscle creatine kinase by phosphorylation in normal and diabetic hearts. *Cell Mol Life Sci.* 2009;66:135-44.
220. Ingwall JS. Is creatine kinase a target for AMP-activated protein kinase in the heart? *J Mol Cell Cardiol.* 2002;34:1111-20.
221. Hammerschmidt S, Bell M, Buchler N, et al. Acute changes of myocardial creatine kinase gene expression under beta-adrenergic stimulation. *Biochim Biophys Acta.* 2000;1502:471-80.
222. Ebermann L, Piper C, Kuhl U, et al. Impact of myocardial inflammation on cytosolic and mitochondrial creatine kinase activity and expression. *Basic Res Cardiol.* 2009;104:247-57.
223. Mitani S, Okumura K, Matsui H, et al. Insulin alters cardiac muscle creatine kinase activity. *Heart Vessels.* 2000;15:23-9.
224. Stockebrand M, Sauter K, Neu A, et al. Differential regulation of AMPK activation in leptin- and creatine-deficient mice. *FASEB J.* 2013;27:4147-56.
225. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med.* 2002;8:1288-95.
226. Schutten MTJ, Houben AJHM, de Leeuw PW, et al. The Link Between Adipose Tissue Renin-Angiotensin- Aldosterone System Signaling and Obesity-Associated Hypertension. *Physiology.* 2017;32:197-209.
227. Redman LM, Smith SR, Burton JH, et al. Metabolic Slowing and Reduced Oxidative Damage with Sustained Caloric Restriction Support the Rate of Living and Oxidative Damage Theories of Aging. *Cell Metab.* 2018;27:805-815 e4.
228. Pope GD, Finlayson SR, Kemp JA, et al. Life expectancy benefits of gastric bypass surgery. *Surg Innov.* 2006;13:265-73.
229. Persson CE, Bjorck L, Lagergren J, et al. Risk of Heart Failure in Obese Patients With and Without Bariatric Surgery in Sweden-A Registry-Based Study. *J Card Fail.* 2017;23:530-537.
230. McDowell K, Petrie MC, Raihan NA, et al. Effects of intentional weight loss in patients with obesity and heart failure: a systematic review. *Obes Rev.* 2018.

231. Breathett K, Allen LA, Udelson J, et al. Changes in Left Ventricular Ejection Fraction Predict Survival and Hospitalization in Heart Failure With Reduced Ejection Fraction. *Circ Heart Fail.* 2016;9.
232. Zhang L, Jaswal JS, Ussher JR, et al. Cardiac insulin-resistance and decreased mitochondrial energy production precede the development of systolic heart failure after pressure-overload hypertrophy. *Circ Heart Fail.* 2013;6:1039-48.
233. Sankaralingam S, Abo Alrob O, Zhang L, et al. Lowering Body Weight in Obese Mice With Diastolic Heart Failure Improves Cardiac Insulin Sensitivity and Function: Implications for the Obesity Paradox. *Diabetes.* 2014.
234. Lavie CJ, Ventura HO,Messerli FH. Left ventricular hypertrophy. Its relationship to obesity and hypertension. *Postgrad Med.* 1992;91:131-2, 135-8, 141-3.
235. Kelley CP, Sbrocco G,Sbrocco T. Behavioral Modification for the Management of Obesity. *Prim Care.* 2016;43:159-75, x.
236. Maciejewski ML, Arterburn DE, Van Scoyoc L, et al. Bariatric Surgery and Long-term Durability of Weight Loss. *JAMA Surg.* 2016;151:1046-1055.