Cardiac Function and Obesity: Effects of Weight Loss

By

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DECLARATION

I declared that this dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

The work describe here not been submitted previously for any degree, diploma or other qualification at any university or institution.

The dissertation does not exceed the work limit of the Degree Committee.

Khin Swe Myint

August 2010

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ABBREVIATIONS

ACCI	Addenbrookes centre of clinical investigation
ADP	Air displacement plethysmography
AG ratio	Android to Gynoid ratio
ATP III	Adult Treatment Panel III
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BNP	B- type natriuretic peptide
BP	Blood pressure
CHD	Coronary heart disease
CO	Cardiac output
CO_2	Carbon dioxide
CPET	Cardiopulmonary exercise testing
CRF	Clinical Research Facility
CRP	C-reactive protein
CVD	Cardiovascular disease
DXA	Dual Energy X-ray Absorptiometry
ECS	Endo-cannabinoid system
EDV	End-diastolic volume
ELISA	Enzyme linked immuno-solvent assay

ESV	End-systolic volume
Fe^{++}	Ferrous ion
Fe^{+++}	Ferric ion
FFM	Fat free mass
FM	Fat mass
FRAP	Ferric reducing ability of plasma
GLP-1	Glucagon like peptide-1
HDL-C	High-density lipoprotein cholesterol
HF	High frequency
HNR	Human Nutrition Research
HRV	Heart rate variability
hsCRP	Highly sensitive CRP
IDF	International Diabetes Federation
IL-6	Interleukin-6
LCD	Low calorie liquid diet
LDL-C	Low-density lipoprotein cholesterol
LELD	Low energy liquid diet
LF	Low frequency
LV	Left ventricle
LVEF	Left ventricular ejection fraction
MET	metabolic equivalence

MRI	Magnetic resonance imaging
NCEP	National Cholesterol Education Programme
NE	Norepinephrine
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NPR-C	Natriuretic peptide clearance receptor
NT-proBNP	Aminoterminal (NT)-proBNP
NYHA	New York Heart Association
O_2	Oxygen
PAI-1	Plasminogen activator inhibitor-1
pVO_2	Peak oxygen consumption
QMR	Quantitative magnetic resonance
ROS	Reactive oxygen species
SNS	Sympathetic nervous system
SVR	Systemic peripheral resistance
TAS	Total antioxidant status
TG	Triglyceride cholesterol
TNF-α	Tumour necrotic factor- alpha
VCO_2	Carbon dioxide output
VLCD	Very low calorie liquid diet
VO_2	Oxygen uptake/consumption

VE	Minute ventilation
VE/VCO$_2$	Minute ventilation and carbon dioxide output slope
VLF	Very low frequency
VT	Ventilatory threshold
WL	Weight loss
WLM	Weight loss maintenance
%VO$_2$	Percentage oxygen consumption

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ABSTRACT

Introduction

Obesity is one of the growing health concerns worldwide due to its increasing
prevalence and increased associated risk of cardiovascular disease (CVD), heart failure,
diabetes and total mortality. Due to a number of pathological mechanisms, obesity
significantly increases the risk of heart failure and cardiovascular death. Paradoxically,
in patients with established heart failure or high cardiovascular risk, a high body mass
index was associated with a better prognosis and a reduction in the mortality. This has
been shown consistently in epidemiological studies. However, there have not been any
weight loss intervention studies to suggest whether therapeutic weight loss is beneficial
or harmful.

Hypothesis

The hypothesis of this study was that weight loss intervention in obese patients with
heart failure or high CVD risk will improve cardiac performance measured as peak
oxygen consumption (peak VO_2) and reduce biomarkers of cardiac risks.

Primary end point

The primary end point of the study was peak VO_2 following acute weight loss (WL,
week 6) and weight loss maintenance phase (WLM, week16).

Secondary end points

The secondary end points were

- Left ventricular ejection fraction (LVEF), heart rate variability (HRV), resting
  plasma b-type natriuretic peptide (BNP), resting plasma norepinephrine,

antioxidant profile, Alanine aminotransferase (ALT), cholesterol, highly sensitive CRP (hsCRP), fasting plasma glucose and insulin, adiponectin, leptin, tissue necrotic factor-alpha (TNF-α) and interleukin-6 (IL-6), exploratory analysis relating changing in the endpoints to body fat mass (FM) and fat free mass (FFM) changes.

- Comparison of Fat mass measured by 4C model *Vs* quantitative MRI.

Methods

Obese patients with either heart failure or a high cardiovascular risk profile (or both) were investigated in order to explore the effect of acute weight loss and weight loss maintenance on cardiac function and various prognostic indicators. 14 patients were recruited of whom 11 completed the study. WL was induced by a liquid meal based diet (low energy liquid diet of 800-1000 kcal/day), which was provided for 6 weeks followed by a WLM phase of a further 10 weeks with the diet adjusted to individual total energy requirements.

A detailed body composition study by the four compartment model was conducted to assess whether changes in body composition in the group would be different to people with simple obesity i.e., difference in fat Vs fat free mass. Cardiopulmonary exercise testing and echocardiography were performed to assess cardiac function and performance. Sympathetic activity assessments (heart rate variability, plasma and urine catecholamine) and other biomarkers (insulin, adiponectin, B type natriuretic peptide, leptin, sensitive CRP, antioxidant profile) were also measured at baseline and at the end of each phase.

Results

The study demonstrated that acute weight loss and short term weight loss maintenance in patients with heart failure and/or high cardiovascular risks were safe. Mean weight loss of 12 kg±4.6 and 14.2 kg±7.3, p <0.0001 were achieved at the end of acute WL and

WLM maintenance phase respectively. In parallel to the weight loss, a significant loss of both fat mass (8.04 kg±3.23 and 11.2 kg±5.82) and fat free mass (4.17 kg±2.05 and 3.24 kg±3) were seen.

Following the body composition changes, there was a significance increase in the primary end point, peak VO_2 at 2.64 ml/kg/min±3.71 and 2.44 ml/kg/min±2 (14.34% and 13.26% improvement) at the end of acute WL and WLM phases respectively. Significant improvements in haemodynamic parameters and reductions in sympathetic activity were demonstrated. There were mean reductions of 11.1 bpm±12.7 and 10.6 bpm±13.4 in resting heart rate, 22.2 mmHg±17.7 and 11.18 mmHg±19.5 in systolic blood pressure, 106.6ng/l±170.9 and 116.5 ng/l±114.6 in plasma norepinephrine, 270 nmol/24h±184.1 and 100 nmol/24h±173 in 24h urinary norepinephrine and increases of 25.28 ms^2±118.3 and 165.5 ms^2±263 in high frequency and 33.4 ms^2±63.5 and 82 ms^2±62.9 low frequency power of heart rate variability).

In addition, improvements i.e. reduction in level of various metabolic parameters that normally increase cardiovascular risks (fasting plasma glucose, insulin, total cholesterol, triglyceride cholesterol, ALT and tumour necrotic factor-alpha) were also demonstrated.

Paradoxical negative correlations were found in between the pVO_2 changes (ml/kg/min) and the FFM loss(r= -0.688, p=0.019). Similar paradoxical positive correlation was demonstrated in between FM loss and pVO_2 (ml/min) (r=0.793, p=0.004) and pVO_2 (ml/FFM kg/min) (r=0.645, p=0.032).

Conclusion

This thesis aimed to demonstrate whether weight loss or weight loss maintenance was of benefit or harm to patients with heart failure and or high cardiovascular risk. The study

was limited by not having a controlled group and based on pilot data. Nevertheless, the thesis was unique highlighting various aspects of cardiovascular assessment and metabolic risk profile in parallel with weight loss and body composition changes. It demonstrated that in obese patients with heart failure/ high CVD, effective therapeutic weight reduction resulted in a healthier body composition and subsequent improvement in their cardiovascular risk profile. This work will form a basic of larger multicentre randomised controlled trial.

CHAPTER 1

INTRODUCTION

CHAPTER 1:   INTRODUCTION

1.1.   Obesity

1.1.1. Definition of obesity

Obesity is a complex, multi-factorial condition characterised by excess body fat to the point where it poses a significant health risk.[1] Body mass index (BMI)[2] is a widely accepted method of assessing the degree of adiposity. BMI is calculated as the weight (in kg) divided by height (in metres squared) (**Equation 1**). Being overweight and obesity can be classified according to BMI (Table 1.1).[2]

Equation 1   BMI = Body Mass Index = body weight (kg)/ height^2 (m)

Table 1. 1.   Classification of obesity

Range	BMI
Underweight	< 18.5 kg/m^2
Healthy weight	$18.5 – 24.9$ kg/m^2
Overweight	$25.0 – 29.9$ kg/m^2
Obesity	$30.0 - 40$ kg/m^2
Morbid obesity	>40 kg/m^2
Excess weight	≥ 25.0 kg/m^2

Within each range, a mid-weight goal may represent the most appropriate standard because several studies suggest that the risk of mortality increases in both overweight

and underweight people.[3, 4] However, a person may be within the desirable norm for body weight relative to height and frame size, yet still have a high percentage of body fat. Generally, men with more than 25% body fat and women with more than 35% body fat are considered obese. In contrast, body weight can exceed the ideal standard because of large muscle mass; in such a case, the person is not considered obese.

1.1.2. Types of obesity

Two patterns of fat distribution have been noted in obese individuals: central abdominal (android obesity) and gluteo-femoral (gynoid obesity). "Android obesity" has been linked to the shape of an apple with the pattern of fat distribution mostly over the trunk whereas "gynoid obesity" is akin to the shape of a pear, with fat distribution mostly around the hips and thighs (Figure 1.1). This terminology was first described by Jean Vague[5] in the 1940s. Android obesity is more closely associated with diabetes, hypertension and cardiovascular diseases than just fatness *per se*. A generally useful method for distinguishing android versus gynoid obesity is to determine waist and hip measurements and calculate a waist/hip ratio. A ratio of less than 0.8 in women and less than 1.0 in men indicates lower cardiovascular risk[6]. A waist circumference exceeding 94 cm in men or 80 cm in women is also considered central obesity. These figures are based on cross-sectional data from Europids and were the best values for identifying people with increased adiposity, defined as a BMI of > 25 kg/m^2 or WHR ≥ 0.90 for men and ≥ 0.85 for women. Therefore, the cut off for waist circumferences is ethnic specific (**See section 1.2.3,** Table 1.4).

Figure 1. 1.   Types of Obesity
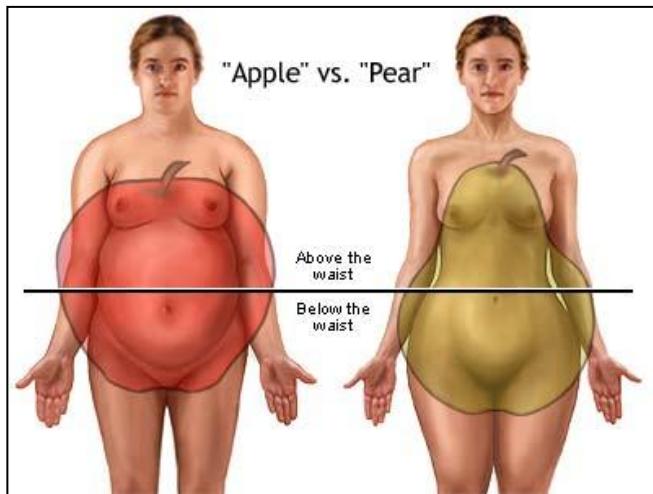
Android obesity (left) demonstrates truncal fat distribution (apple shape) and gynoid obesity (right) demonstrates fat distribution around the gluteo-femoral region (pear shape).

1.1.3. Epidemiology of Obesity

Being overweight and obese are global problems, affecting over one billion adults and 17.6 million children under 5 years of age.[7] It is one of the most rapidly increasing health concerns in the EU as well as globally. Prevalence data from the Europe indicated that 35.7 % of the adult population could be considered overweight (BMI >25 kg/m^2) with a further 17.2 % obese (BMI >30 kg/m^2), resulting in over 53% of the European population being classified as either obese or overweight.[8] The recent Health Survey for England[9] showed that one in four adults was obese in 2004. The prevalence had risen from 13% to 24% in males (40% rise) and 16% to 24% in females (28% rise) compared with that of 1993. In addition, the prevalence of obesity among children and adolescents is rapidly increasing. In the UK, around 5% of boys and 11% of girls aged 11 to 15 years were considered obese according to International Obesity Task Force (IOTF) definitions.[10] However, if a more commonly used definition from 1990 Growth Charts

was used, the prevalence was higher at 25% (overweight defines percentile of weight-for-length or BMI-for-age $\geq 95^{th}$ and risk of overweight defines that of between 85th and 95th percentile). The degree of obesity had also worsened. Morbid obesity (BMI >40 kg/m^2) had become a considerable health concern. In 1986, 1 in 200 adult Americans were morbidly obese; and this has risen up to 1 in 50 more recently.[11] The rate of increase in BMI >40 was twice as rapid as the rate for BMI >30. Approximately 4% of US males and 6% of females were classified as morbidly obese, while comparable figures for England were 1% and 3% respectively.[12]

The report from the UK government's Foresight project[13] indicated that at current trends, it was projected that 36% of males and 28% females in UK will be obese by 2015, 47% and 36% respectively by 2025, and alarmingly at 60% and 50% respectively by 2050 . By 2021, the proportion of adult males with healthy BMI (<25) was estimated to be 13% whereas the overweight and obese adult males would equally share the remaining 87% of the population (around 43% each) (Figure1.2.). For women, proportions of overweight and obese adults were estimated to reach 70% (35% each) (Figure 1.3).

Figure 1. 2.   Probability of males aged 21–60 belonging to a specific BMI group in a given year

[Dots represent each year data from the Heath Survey of England, curves show predicted future proportion to 2050, shaded area represent 95% confidence limits]
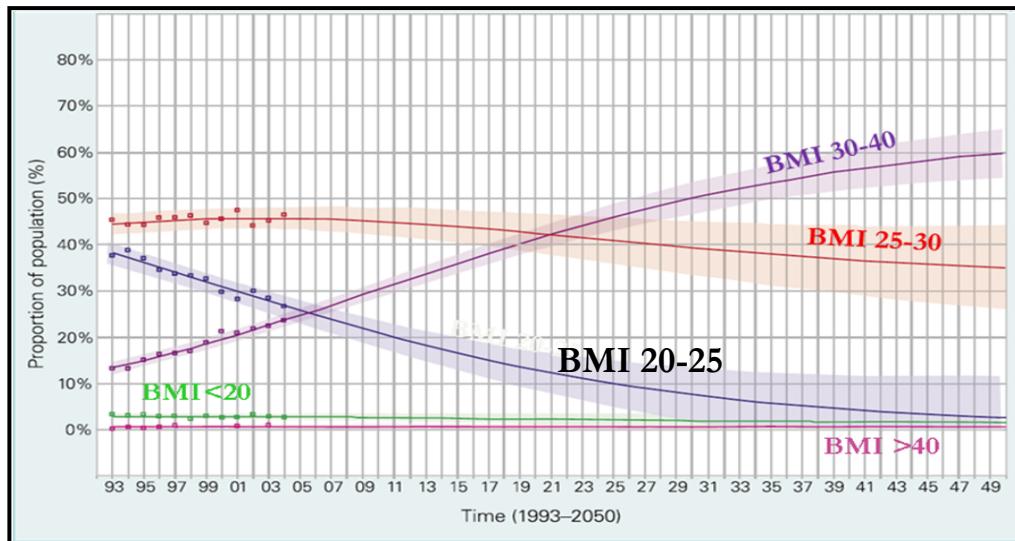
Figure 1. 3.   Probability of females aged 21–60 belonging to a specific BMI group in a given year

[Dots represent each year data from the Health Survey of England, curves show predicted future proportion to 2050, and shaded area represents 95% confidence limits]

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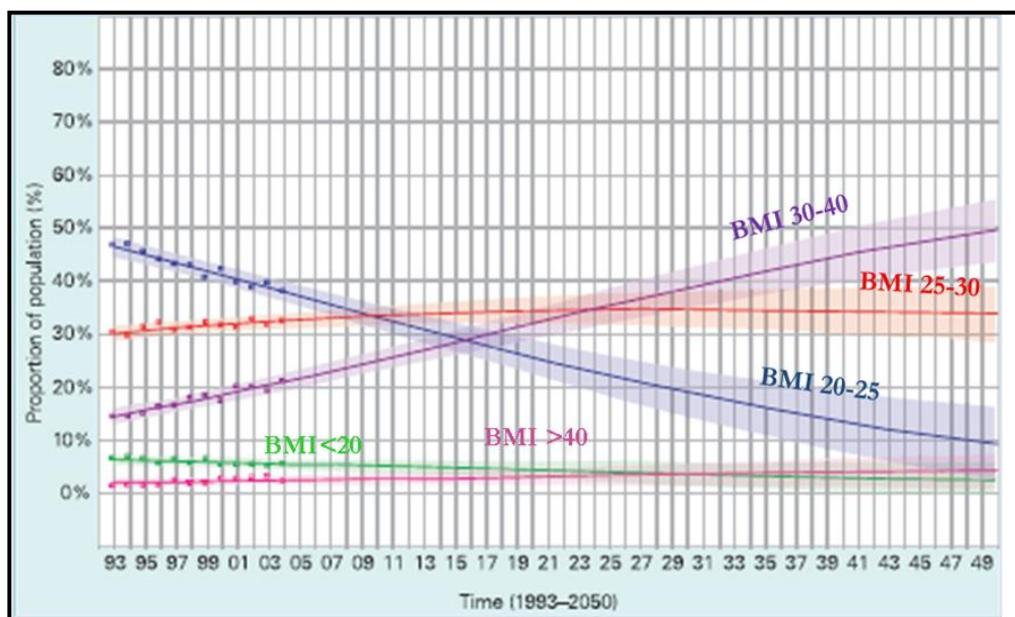
1.2. Obesity and associated health risk

Health risks are increased with increasing BMI. A BMI > 30 kg/m^2 increases the risk of co-morbidities and mortality exponentially.[14]

1.2.1. Mortality

Obesity is strongly associated with all cause mortality.[15-17] Adams et al[18] conducted a large prospective study of 520 000 people aged 50 to71 years over a 10 year period. The study examined the risk of death in relation to BMI after adjusting for age, race or ethnic group, level of education, smoking status, physical activity and alcohol intake. Excess weight accounted for approximately 7.7% and 11.7% of all premature deaths in men and women respectively. The association was much higher in people who never smoked (18.1% for men and 18.7% for women). The risks of death amongst obese individuals were two to three folds of that of BMI 23.5 to 24.9 whereas in overweight individuals it was 20 to 40% higher. BMI >40 kg/m^2 was associated with an increased risk of sudden death.[19]

1.2.2. Cardiovascular disease and heart failure

Obesity is known to exert numerous adverse effects on cardiac function.[20, 21] It is associated with an increased risk of essential hypertension,[22, 23] stroke,[24] peripheral vascular disease, coronary heart disease,[25, 26] heart failure[25, 27] and death.[28-30] Despite the common belief that simple obesity without any cardiovascular risk is a benign condition, there is evidence that obesity is an independent risk factor for CVD.[29, 31] Mortality due to CVD is almost 50% higher in obese patients than in those of average weight and is 90% higher in those with severe obesity.[32] Obese children have also been documented as having high CVD risks.[33, 34]

Heart failure is a clinical syndrome characterised by inadequate systemic perfusion to meet the body's metabolic demands as a result of impaired cardiac pump action. In

systolic heart failure, there is reduced cardiac contractility and hence reduced left ventricular ejection fraction, whereas in diastolic heart failure, there is reduced cardiac relaxation and abnormal ventricular filling. In 1928, the New York Heart Association (NYHA) classified heart failure according to clinical severity and prognosis (Table 1.2).[35] It is based on the functional capacity of the patients.

Table 1. 2.   New York Heart Association (NYHA) classification of heart failure.

Class I:	No limitation is experienced in any activities; there are no symptoms from any ordinary activity
Class II:	Mild limitation of activity; patient is comfortable at rest or only mild exertion but symptomatic on moderate exertion
Class III:	Marked limitation of any activity; patient is comfortable at rest
Class IV:	Any physical activity brings on discomfort and symptoms occur at rest.

More recently, obesity has been shown to be strongly and independently associated with an increased risk of heart failure.[25, 27, 36] Kenchaiah et al[36] have studied the relationship between BMI and the incidence of heart failure among 5881 participants from the Framingham Heart study over 14 years. With each increment of BMI, the risk of heart failure rose 5 and 7% in men and women respectively after adjustment for established risk factors. Overweight women had a 50% increased risk of heart failure, and obese women were twice as likely to develop heart failure compared with normal weight individuals. Overweight and obese men had a 20% and 90% increase risk respectively of developing heart failure.

The pathophysiology of heart failure in obese individuals is complex. Obesity is a major risk factor for developing hypertension, diabetes and left ventricular hypertrophy that in turn contribute up to 90% of the risk of developing heart failure.[37] The structural and

haemodynamic alteration, in conjunction with hypertension, coronary heart disease, obesity cardiomyopathy, insulin resistance syndrome, increases the risk of heart failure in obesity.

Effects on ventricular function and haemodynamic changes

Obesity produces a variety of structural and haemodynamic changes to the myocardium. In early obesity, excess body weight increases metabolic demand and the subsequent expansion in total intravascular volume[38] results in an increase in cardiopulmonary volume or pre-load. Therefore, the cardiac workload is greater at any given level of activity in obese subjects. Increased stroke volume is a major attributable factor for the higher cardiac output seen in these individuals. Because of the high right ventricular filling pressure and volume, the Starling curve is shifted to the left (Figure 1.4). Over time, this may result in dilatation of the ventricular chamber therefore predisposing to increased myocardial mass and development of eccentric left ventricular hypertrophy (LVH).[39, 40] A long history of obesity, with an increase in left ventricular mass, is associated with both systolic and diastolic dysfunction leading to a propensity for more complex ventricular dysrhythmias. These early abnormalities as well as impairment in both diastolic and systolic ventricular function, can be reversed following marked, purposeful weight reduction.[41]

Figure 1. 4.   Frank Starling curves
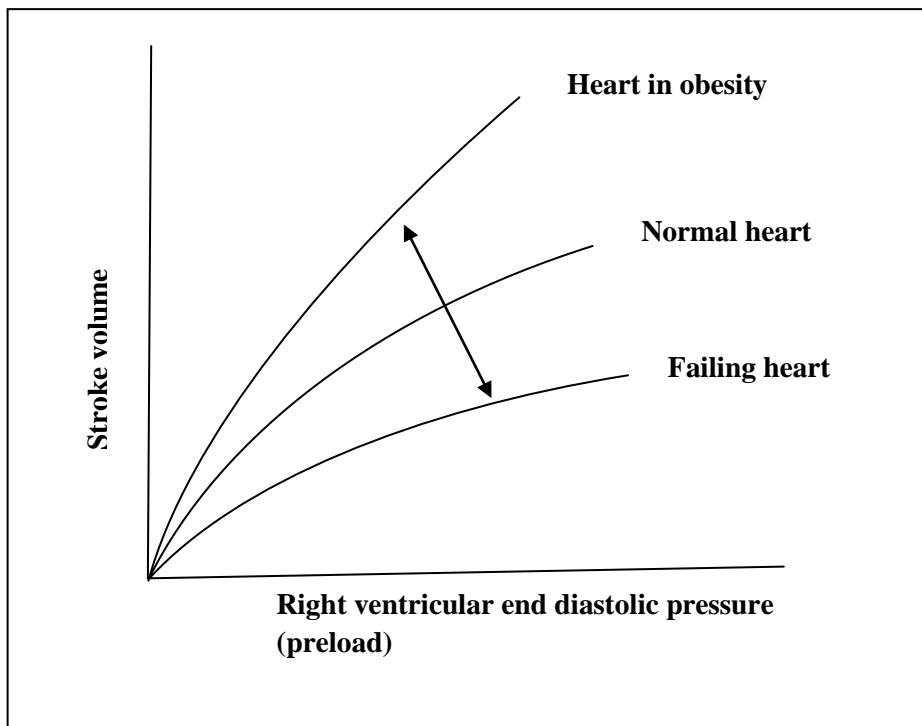
Cardiomyopathy of Obesity

 The development of obesity related cardiomyopathy has been recognised since 1818 when Cheyne described a historic case in which he observed a fatty heart as well as Cheyne-Stokes respiration in an obese patient.[42] Autopsy revealed an increased heart weight and left and right ventricular hypertrophy in proportional to the degree of obesity.[43] The pathognomonic histological feature of obesity related cardiac pathology is diffused myocyte hypertrophy.[44] Fatty infiltration of the myocardium and excessive epicardial fat deposition are also closely associated with the duration of obesity.[45, 46] It was observed that the initial fatty deposit is most likely due to metaplasia rather than infiltration.[47] This may represent an adaptive response to the environment by substituting sensitive cells with more resistant ones to enduring stress. The intracellular accumulation of fat in between muscle fibres results in myocyte degeneration leading to cardiac conduction defects.[48]

In addition to those structural and histological changes, severe obesity has been found to be associated with idiopathic dilated cardiomyopathy. Kasper et al[49] compared patients with a diagnosis of congestive cardiac failure (49 patients with marked obesity defined by BMI >35 kg/m^2 and 409 lean) where idiopathic dilated cardiomyopathy was diagnosed as the cause of heart failure in 77% obese individuals compared with <35% of lean subject (p<0.001). In a reported case series, dilated cardiomyopathy was the most common cause of death in 22 severely obese individuals (average weight 175kg) due to sudden cardiac death.[50] Fatal arrhythmia was a frequently cause of death in these individuals.[51] The mechanism of obesity cardiomyopathy is multifactorial.[52] The most important mechanisms are metabolic disturbances (section 1.2.3), activation of the renin angiotensin aldosterone system, sympathetic nervous systems, haemodynamic changes and geometric remodelling and endothelial dysfunction.[43]

Coronary heart disease (CHD)

Obesity and being overweight have long been known as risk factors for the development of CHD. Anderson et al reviewed 11 studies to investigate the relationship of body weight and CHD risk.[53] The relative risks of developing CHD were 2.72 and 2.8 times higher in obese women and men respectively compared to non-obese subjects. Obesity is the major contributor to dyslipidaemia, hypertension, diabetes that are in turn major risk factors for developing CHD.

Obesity, particularly visceral adiposity, is strongly associated with a typical dyslipidaemic pattern: elevated low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) and reduced high-density lipoprotein cholesterol (HDL) level[54] (see detail on **section 1.2.3**). An elevated total cholesterol level is an established as risk factor for CHD.[55, 56] There is increasing evidence to suggest that CHD is a manifestation of a chronic inflammatory response to injury or infection. The normal endothelium can be disrupted by either infection or injury leading to the formation of fatty streaks or early atherosclerosis.[57] The early signs of atherosclerosis are deposition of cholesterol ester in monocyte–derived macrophage foam cells in the intima of large muscular

arteries.[58, 59] This process begins in early life (5-10 years old). With time, the atherosclerotic lesion becomes more complex. Activated foam cells release chemoattractant molecules, cytokines, and growth factors. This attracts more lymphocytes to the lesion, and in turn, add to the pool of effector molecules that expand and perpetuate the inflammatory response.[57] As this cycle is repeated, the plaque develops a fatty core covered by a fibrous matrix that stabilizes the structure.[57] There is emerging evidence that 70% of all fatal acute myocardial infarctions and sudden coronary deaths are attributable to plaque rupture[60, 61] or plaque erosion.[62]

In addition to dyslipidaemia, obesity also increases the risk of developing hypertension. In patients with hypertension, the concentration of angiotensin II, the principle product of the renin angiotensin aldosterone system, is often elevated. Angiotensin II not only is a potent vasoconstrictor but also stimulates the growth of vascular smooth muscles and hence contributing to atherogenesis.[63] Angitensin II, by binding to the specific receptor of smooth muscle, activates phospholipase C resulting in intracellular calcium accumulation and hence increases smooth muscle contraction, protein synthesis and smooth muscle hypertrophy.[64] Together with dyslipidaemia, angiotensin II has synergistic effects generating reactive oxygen species (ROS) in the vessel wall.[65] Angiotensin II increases smooth-muscle lipoxygenase activity, and subsequently increases inflammation and the oxidation of LDL.

Lowering cholesterol levels, particularly LDL-C, has been the focus of preventing CHD and its sequealae for almost 25 years.[66] Evidence suggests that lipid-lowering therapy reduces inflammation, which may reduce the risk of cardiovascular events, even for individuals with normal LDL-C levels (<130 mg/dL or 3.4mmol/l) based on the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines.[67] More recently Ridker et al[68] has studied a 17802 healthy individuals with normal LDL-C (< 130mg/dL or 3.4 mmol/l) but elevated high-sensitive C-reactive protein (hsCRP), a marker of inflammation, randomly assigned to rosuvastatin or

placebo. They have demonstrated that in rosuvastatin treated group, there were significant reduction in incidence of major cardiovascular events including myocardial infarction (hazard ratio, 0.46; 95% CI, 0.30 to 0.70; P=0.0002), 0.18 and 0.34 for stroke (hazard ratio, 0.52; 95% CI, 0.34 to 0.79; P=0.002), 0.41 and 0.77 for revascularization or unstable angina (hazard ratio, 0.53; 95% CI, 0.40 to 0.70; P<0.00001), 0.45 and 0.85 for the combined end point of myocardial infarction, stroke, or death from cardiovascular causes.

Hypertension

Hypertension is 6 times more frequent in obese subjects than lean individuals.[69] 60% of obese individuals suffer from hypertension.[45] Weight gain in young individuals is a significant risk factor for subsequent development of hypertension. Every10kg of excess body weight is associated with a 3/2.2 mmHg rise in systolic and diastolic blood pressure respectively.[70] This implies an estimated 12% increased risk of CHD and 24% increase risk of stroke.[71] Weight loss in obese and overweight patients has showed to be an effective measure to reduce blood pressure. Neter et al[72] conducted a meta analysis of 24 randomised controlled trials wherein a 5kg weight loss resulted in a 4.4/3.6 mmHg reduction in blood pressure.

Arrhythmia

Obesity confers an increased risk of arrhythmias and sudden cardiac death, even in the absence of cardiac dysfunction. In the Framingham study, obese men and women had increased mortality due to unexplained sudden cardiac arrest. This was estimated to be 40 times higher in obese than non obese individuals.[73] QT_c interval prolongation is observed in relatively higher percentage in obese subjects and the association is more pronounced in severely obese individuals (about 8% had QT_c of >0.44 seconds and 2% with QT_c of >0.46 seconds).[74] The clinical significance of obesity-associated prolonged QT interval remains unclear. Elevated levels of circulating free fatty acid in obese individuals may affect the cardiac repolarisation partly due to higher plasma

catecholamines.[75] Hyperglycaemia may reduce nitric oxide (NO) availability leading to increased vasomotor tone and ventricular instability.[76] In addition, as previously stated dilated cardiomyopathy is often seen in morbidly obese subjects and fatal arrhythmias is the frequent cause of death.[51]

Stroke

Obesity is a potential modifiable risk factor for stroke since it increases the risk of diabetes, dyslipidaemia and hypertension. In a recent cohort of 21 414 men, obese men were shown to have a significantly higher multiple adjusted relative risk of developing stroke. (2.00 for stroke, 1.95 for ischaemic stroke, and 2.25 for haemorrhagic stroke).[77]

1.2.3. Metabolic syndrome and diabetes

Metabolic syndrome is defined as the constellation of metabolic risk factors including central obesity, atherogenic dyslipidaemia, hypertension and insulin resistance (impaired glucose tolerance, impaired fasting glucose, type 2 diabetes and hyperinsulinaemia). The cluster of obesity, dyslipidaemia, hypertension and diabetes has been noted since mid 1960s.[78] The link between this cluster and the insulin resistance was first described by Professor Reaven at Stanford in his Banting lecture in 1988.[79] He called this entity "Syndrome X", later to be termed metabolic syndrome. [80-82] The World Heath Organization,[83] European Group for study of insulin resistance (ERIG) and NCEP/ATP III[54] have since separately developed different criteria to define the metabolic syndrome. More recently, the International Diabetes Federation has developed a new consensus statement for criteria of metabolic syndrome (Table1.3).[80]

Table 1. 3.   Definition of metabolic syndrome (The International Diabetes Federation)

Central obesity	Waist circumference—ethnicity specific plus any two of the following:
Raised triglycerides	≥ 1.7 mmol/l or specific treatment for this lipid abnormality
Reduced HDL cholesterol	1.03 mmol/l in males < 1.29 mmol/l in females or specific treatment for this lipid abnormality
Raised blood pressure	Systolic: ≥ 130 mmHg or Diastolic: ≥ 85 mmHg or treatment of previously diagnosed hypertension
Raised fasting plasma glucose	$\geq$ Fasting plasma glucose ≥ 5.6 mmol/l or previously diagnosed Type 2 diabetes. If > 5.6 mmol/l, oral glucose tolerance test is strongly recommended but is not necessary to define presence of the syndrome

Central obesity is the key component of the new definition of metabolic syndrome and most easily measured by waist circumference with cut-points that are gender and ethnic-group specific (Table 1.4).

Table 1. 4.   Country/ethnic-specific values for waist circumference

Country/ethnic group	Waist circumference (as measure of central obesity)
Europids	Male ≥ 94 cm, Female ≥ 80 cm
South Asians	Male ≥ 90 cm, Female ≥ 80 cm
Chinese	Male ≥ 90 cm, Female ≥ 80 cm
Japanese	Male ≥ 90 cm, Female ≥ 80 cm
Ethnic South and Central Americans	Use South Asian recommendations
Sub-Saharan Africans	Use European data
Eastern Mediterranean and Middle East	Use European data

The pathogenesis of metabolic syndrome is complex and has yet to be fully evaluated. However, it is thought that central obesity and insulin resistance are the key culprits in the development of metabolic syndrome leading toward to a prothrombotic and proinflammatory state.[80-82]

Obesity is a critical determinant of dyslipidaemia, operating through a number of metabolic influences that include reduced insulin sensitivity and changes in fatty acid metabolism. Variations in the nature and magnitude of the dyslipidaemia are due to the interaction of genetic factors with environmental influences, most notably diet and physical activity, and possibly stress.[84] Previously, adipocytes were considered to be

little more than inert storage depots, storing fat as triglyceride in the fed state, and releasing fuel as fatty acids and glycerol in times of fasting. It is now clear that adipocytes are dynamic endocrine glands as well as an active metabolic cells that secrete important hormones, cytokines and vasoactive substances; tumour necrosis factor alpha (TNFα), leptin, plasminogen activator inhibitor-1 (PAI-1), interleukin-6 (IL-6), resistin, and angiotensinogen.[85] These exert marked influences on metabolic function and cardiovascular risk in a number of organ systems throughout the body. PAI-1 being an endogenous inhibitor of tissue plasminogen activator (tPA), increases the risk of intravascular thrombus formation and plays a key role in promoting thrombosis in a ruptured coronary plaque.[86] IL-6 and TNFα are inflammatory mediators. Intravascular inflammation is a key event in atherogenesis. IL-6 stimulates the production of C-reactive protein (CRP) from the liver. CRP is an important marker of vascular inflammation and predictor of atherogenesis.[87] Angiotensinogen is a precursor of angiotensin II, a potent vasoconstrictor, and activates the renin angiotensin aldosterone system. In 1998, leptin was shown to enhance cellular immune responses,[88] as well as increase blood pressure.[89] Leptin decreases insulin sensitivity when given to obese rats. Adiponectin is distinct from other adipokines in that it improves insulin sensitivity and inhibits vascular inflammation.[90]

Obesity is the most powerful environmental risk factor for type 2 diabetes. A strong positive correlation between obesity and the relative risk of diabetes has been established in both men[91] and women.[92] Men with a BMI of ≥ 35 kg/m^2 have a multivariate relative risk of 42.1 compared with men with a BMI < 23.0 kg/m^2. The age adjusted relative risk is greater in women, with a nearly 100-fold increase in the risk of developing diabetes if BMI is ≥ 35 kg/m^2 compared those with BMI is <22 kg/m^2. The risk of developing diabetes can be minimised by having a lean body weight on entering adulthood and avoiding even modest weight gain throughout life.

1.2.4. Non alcoholic fatty liver disease (NAFLD)

Non alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver damage, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis.[93] Obesity and insulin resistance are strongly linked to NAFLD.[94, 95] In an autopsy series of 351 patients, 70% of obese patients had hepatic steatosis and the degree of steatosis was proportional to the degree of obesity.[96] In a study of 100 morbidly obese patients who underwent Roux-en-Y gastric bypass surgery, 98% of the patients were found to have histological changes in the liver ranging from mild fatty infiltration to severe cirrhosis.[97] Insulin resistance is a major contributing factor to the development of NAFLD in obese subjects.[94, 95] It has recently been suggested that visceral adiposity and insulin resistance promote the hepatic influx of free fatty acid resulting in increased triglyceride synthesis and decrease hepatic triglyceride export leading to hepatic steatosis. A small proportion of these patients developed NASH.

1.2.5. Other Co-morbidities

There has been growing evidence that the adverse outcome of obesity is not limited to CVD but affect on various health issues.[98, 99]

- Malignancy

Mortality from cancer among non-smoking obese people is elevated by around 40% compared to non-obese people.[100] Compared to women with normal weight, obese women have higher mortality rate from certain malignancies including endometrium, cervix, ovary, gallbladder and breast (post menopausally).[101] Obese individuals with BMI >35kg/m^2 have increased mortality from colonic cancer.[102]

- Mechanical complication

Obesity is associated with many less serious but debilitating conditions such as shortness of breath, back pain,[103-106] reduced mobility and poor quality of life, as well as an increased psychological and social burden.

- Endocrine or other metabolic abnormalities.

Obesity is frequently associated with hyperandrogenism in women because hyperinsulinaemia causes increased ovarian androgen secretion. This may result in hirsutism, anovulatory cycles, and decreased fertility.

- Sleep apnoea[107-109]

Obesity also is an underlying cause of sleep apnoea, 50 to 75% of those patients are obese. Sleep apnoea is associated with traffic accidents, cardiovascular disease and stroke. It also associated with raised pulmonary arterial pressure and pulmonary hypertension (in 25% of patients).

1.3.   Prognostic indicators of cardiovascular mortality in heart failure

There are established various echocardiographic measurements, physiological parameters and biomarkers being used as prognostic indicators for cardiovascular mortality.[110]

1.3.1. Left ventricular ejection fraction

Left ventricular ejection fraction (LVEF) is recommended by the American Heart Association as routine assessment of patients with heart failure to guide therapy. It can be measured by two-dimensional echocardiography, equilibrium radionuclide angiography, left ventricular contrast angiography or more recently with cardiac MRI.[111] LVEF is a well established prognostic indicator of prognosis in heart failure patients.[112, 113] Curtis et al[111] studied 7788 patients with heart failure with a median follow up of 37 months. In patients with LVEF $\leq$ 45%, all cause mortality decreased in a near linear fashion across successively higher LVEF groups (LVEF < 15% at 51.7%; LVEF 36% to 45% at 25.6%; p < 0.0001).

1.3.2. Peak oxygen uptake and anaerobic threshold

Measurement of oxygen uptake (VO_2) in patients with chronic heart failure was first described by Weber et al[114] as a non invasive method for characterising cardiac reserve and cardiac functional status in these patients. Both peak VO_2 and aerobic threshold (AT) measurement during cardiopulmonary exercise testing has proved to be an objective, reproducible, safe, and non-invasive method for characterizing cardiac reserve.[115] VO_2 can be influenced by the O_2 carrying capacity of the blood (available haemoglobin and the arterial O_2 saturation, dissociation shift curve with temperature and carbon dioxide), cardiac function, peripheral blood flow and tissue perfusion.[116] VO_2 increases linearly during incremental exercise until it is limited by one or more of the determinants (stroke volume, heart rate or tissue extraction) and VO_2 versus work rate then may begin to plateau. VO_2 at plateau is called maximum VO_2 (VO_2 max). However, a clear plateau can be difficult to achieve because of the symptom limitation of exercise. Therefore, peak exercise oxygen consumption measured during maximal exercise testing (pVO_2) is more frequently used as an estimate of VO_2 max. Decreased peak VO_2 or aerobic capacity is associated with increased cardiovascular disease and all-cause mortality.[117, 118] Peak VO_2 is a strong predictor of mortality and is commonly used in the evaluation of patients to determine the severity of heart failure and is an indication for cardiac transplantation.[110, 115] Heart failure patients with peak VO_2 >14 ml/kg/min had a relatively better prognosis compared to those with a lower peak VO_2. The prognosis was similar to those who had cardiac transplant (approximately 90% at one year) and cardiac transplantation in this particular group would not improve their outcome. Peak VO_2 of 14 ml/kg/min has therefore been widely used as a cut off value for cardiac transplantation.

1.3.3.   Minute ventilation and carbon dioxide output slope

The relationship between minute ventilation and carbon dioxide output (VE/VCO_2) is part of the measurement derived during cardiopulmonary exercise testing. During exercise, there is a linear relation between ventilation (VE) and the CO_2 production. The slope of the relation between the two represents the ventilatory response. In patients with chronic heart failure, there is greater ventilation for a particular CO_2 production rate indicated by an increased VE/VCO_2 slope (Figure 1.5). This VE/VCO_2 slope has recently been demonstrated as a prognostic indicator for heart failure.[119, 120] It has consistently shown that a high slope is a significant predictor of mortality[119-121] and hospitalisation[122] in heart failure patients.

Figure 1. 5.   Example of VE/VCO2 slope in normal, mild heart failure and moderate heart failure[123]
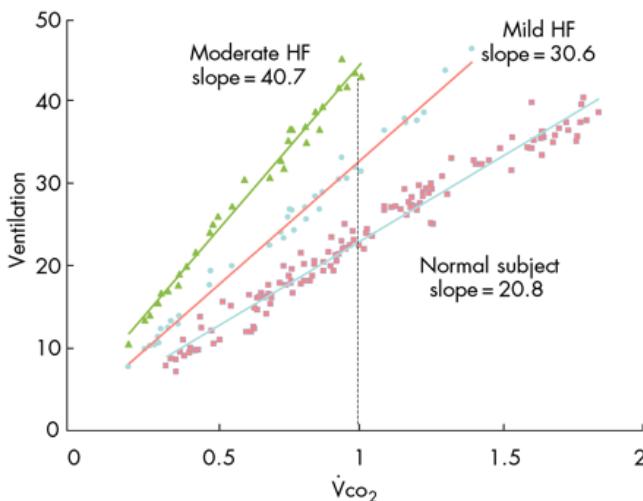
1.3.4.   B- type natriuretic peptide

B- type natriuretic peptide (BNP) is a cardiac neurohormone with 32 amino acid residues secreted from the atria and ventricles. It is released as a proBNP and then enzymatically cleaved to N-terminal-proBNP and BNP in response to the ventricular wall stretch or dilation, and/or increased cardiac chamber pressures resulting from fluid

overload (Figure 1.6). Increased BNP secretion causes vasodilatation, increased sodium excretion, and diuresis, thus lowering blood volume and arterial blood pressure. Physiological responses to, or associated with, exercise, posture, and diuretics, angiotensin converting enzyme inhibitor, adrenergic agonist, sodium intake, thyroid hormones and glucocorticoids are linked with increases in circulating BNP.[124] Levels also are increased with age, female gender, and during daytime compared to night.

Plasma BNP level is a sensitive and specific marker of left ventricular dysfunction (LVD) with 73% sensitivity and up to 85% specificity for echocardiographic mild LVD.[125] Falls in circulating BNP are associated with increased EF and ventricular volumes, and therefore may serve as a useful marker of ventricular remodelling such as that seen in progressive heart failure.[126] In patients with stable chronic heart failure, a rise in BNP together with a fall in percent VO_2 is a sign of poor prognosis. de Groote et al[127] showed a high BNP level (>109 pg/ml) alone, was a predictor of reduced survival. In heart failure patients, each 100 pg/ml increase of BNP was associated with a 35% increase in the relative risk of death.[128]

The relationship between BNP and obesity has been investigated in several recent studies and has confirmed that, similar to the non-obese, there is an inverse relationships between BMI and BNP concentration in subjects with[129] or without[130] heart failure. Das et al[131]confirmed the association inverse correlation between BMI and a BNP level, that remained when LBM replaced BMI. The relative importance of the increased FFM or LBM in obesity in accounting for low BNP levels is therefore not known.

 Investigating plasma BNP levels as a marker of cardiac function in obese patients with heart failure and its response to therapeutic weight loss is therefore of theoretical and clinical interest.

Figure 1. 6.   Human BNP , pro-BNP and NT-proBNP [132]
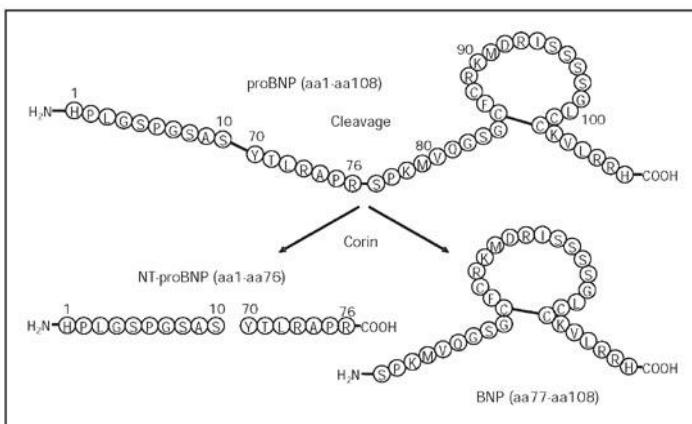
B-type natriuretic peptide (BNP) is produced as pre–prohormone BNP (pro-BNP), processed to pro-BNP, and then cleaved by corin to mature, biologically active 32–amino acid (aa) BNP and non–biologically active N-terminal (NT)-pro-BNP.

1.3.5. Sympathetic nervous system activation: Plasma norepinephrine

The catecholamines: Norepinephrine (NE), epinephrine and dopamine; are synthesized in the central nervous system, sympathetic nervous system and chromaffin cells of adrenal medulla. In 1946, NE was first isolated and identified in the sympathetic nervous system by Von Euler[133] demonstrating that sympathetic neurons use NE, rather than epinephrine, as a neurotransmitter. He later developed sensitive methods for measuring tissue norepinephrine.[134] In 1954, Vogt[135] demonstrated that NE was present in the brain. The biosynthesis pathway of catecholamine was proposed by Blaschko[136] in 1939, and it was finally established by Udenfriend and colleagues.[137] The precursor of catecholamine is the amino acid L-tyrosine which is ingested with NE food or synthesized in the liver from phenylalanine. All biosynthesis reactions occur within the sympathetic nerve terminal apart from the final step that occurs exclusively in the adrenal medulla. This step is catalyzed by phenylethanolamine-N-methyltransferase (PNMT). The NE released from the nerve ending either bind to the post-synaptic receptor (adrenergic receptor) for its action or reuptake by the pre-synaptic terminal.

The adrenergic receptors can be subdivided into α (α_1 and α_2) and β (β_1 and β_2) adreno-receptors. These receptors are coupled by G proteins to the various effector proteins and subsequent cascade of reaction giving rise to a series of sympathetic activities.

1.3.6. Sympathetic nervous system in cardiovascular disease

In congestive heart failure, sympathetic nervous system activation is one of the important pathophysiological mechanisms evidenced by having higher plasma concentration of NE.[138] It was suggested that heart failure patients have an increased release of norepinephrine into the plasma and reduced renal clearance from plasma (higher cardiorenal spillover of NE) compared with subjects with normal cardiac function.[139] In addition, plasma NE concentration is significantly and independently correlated with mortality in congestive heart failure. In a study by Cohn et al,[140] plasma NE level below 600 pg/ml were uniformly associated with better than average survival and values above this level were associated with poorer prognosis.

It has been recognised that there is a significant relationship between autonomic nervous system activity and cardiovascular mortality, including sudden cardiac death in both ischaemic cardiomyopathy[141] and chronic heart failure.[142] Experimental evidence has shown an association between a propensity of lethal arrhythmias and signs of either increased sympathetic activity or reduced vagal activity.[143] Heart rate variability (HRV) is a commonly used method of accessing autonomic function (See detail in Chapter 2). Bilchick et al[141] has demonstrated that in a group of patients with congestive cardiac failure, low HRV by the measure of standard deviation of normal- normal interval (SDNN) of <65.3 ms was the useful predictor of sudden cardiac death and survival. Similar findings were also found in patients with a history of myocardial infaction.[144]

1.4.   Weight loss in obese patients with heart failure

Weight loss has been recognized as a key factor in the control and prevention of coronary heart disease, hypertension, type 2 diabetes, dyslipidaemia, cardiorespiratory failure and other chronic degenerative diseases in obese patients.

As previously stated, an increased BMI is associated with increased risk of heart failure. Paradoxically, several studies have showed that obese patients with heart failure seem to have a more favourable clinical prognosis than non-obese counterparts. Lavie et al[145] investigated 209 ambulatory patients with chronic heart failure retrospectively looking at their body composition by means of weight and skin fold thickness in relation to clinical events (cardiovascular death or urgent transplantation). Patients in the higher quartile for BMI had greater event-free survival. Every 1% absolute increase in percent body fat was associated with a >13% reduction in major events. However, as the authors' concluded, the study did not answer whether this was causal or merely a speculative association. These findings were in line with a more recent study by Kenchaiah et al.[146] They studied 1831 patients with heart failure over with a median follow-up of 37.7 months. In patients with a BMI $<35 kg/m^2$, a decrease in 1 BMI unit was associated with a 3.3 % increased in all cause mortality adjusted to age and sex. Lower BMI patients had much higher graded risk of fully adjusted all caused mortality in comparison with those with a BMI between 30-35 kg/m^2. It was estimated 22.3%, 44.7% and 69.7% increased risk in BMI group of 25-29.9, 22.5-24.9 and <22.5 kg/m^2 respectively. Kenchaiah et al[27] reviewed 10 studies on the impact of obesity on survival after the onset of heart failure. Most studies suggested an increase in BMI recorded at the time of studies on heart failure was associated with a decline in mortality. However, most of these studies were conducted in patients with advanced heart failure where cardiac cachexia may have contributed to involuntary weight loss, and excess mortality. Anker et al [147] showed that the cachectic state in heart failure patients is an independent predictor of mortality (50% at 18 months).[147] Gustafsson et al[148] retrospectively studied 4700 hospitalised patients with heart failure. In this study, the survival rate was also higher in the obese group compared with the low BMI group, whereas survival rate of

patients with LV systolic dysfunction followed a U-shaped curve with the lowest rate of death in normal weight patients.

In most studies, the duration of heart failure was not taken into account. This may be a critical factor because patients with a longer duration of heart failure have a lower likelihood of survival. The mortality of heart failure is approximately 45% and 60% at 1 and 5 years respectively after diagnosis.[149, 150] More recent statistic[151] from American Heart Association highlighted that based on 44 years follow up of Framingham heart study, 80% men and 70% of women with heart failure and aged <65 years died within 8 years. Finally, most studies only assessed BMI, which may be an inaccurate indicator of total body fat because of fluid retention. One study has looked at the percentage body fat in term of skin fold thickness[152] but this methodology did not give information on the distribution of the fat. No detailed studies of body composition and body fat distribution in heart failure have been carried out.

To date there has not been a study of cardiac function pre and post therapeutic weight loss intervention in obese heart failure patients, there has been an isolated case report in which heart failure was reversed in a case a morbidly obese patient following dietary intervention.[153]

1.5.   Weight loss intervention

1.5.1. Low and very low calorie liquid diet

Very low calorie liquid diets (VLCD) are formulated foods with an energy content of between approximately 450 and 800 kilocalories daily. These foods are intended for use, as presented, except for the addition of water where applicable, as the sole dietary source of energy and all essential nutrients required in excess weight loss programmes. Over the years the definitions of VLCD and low calorie liquid diet (LCD) have changed

with regard to the energy restriction level. In the early days more emphasis was given on the very-low calorie level with values of 250 kcal/d or less. With the introduction of the international CODEX standardization and legislation by the U.S. Food and Drug Administration and the European Union on this type of food restriction,[154] VLCDs are now defined as total diet replacements with <800 kcal and >400 to 450 kcal/d. Diets consisting of between 800 and 1200 kcal/d are classified as LCDs, whereas meal replacements are limited to 200 to 400 kcal. These types of definitions and regulations using fixed energy levels for all users ignore individual differences in body size and thus energy requirements. Therefore, the use of VLCDs and LCDs results in significantly different weight loss for different groups, for instance, between men and women.

The Codex Alimentarius Commission has developed a standard for formula foods for use in VLCD for weight deduction. CODEX was created in 1963 by Food and Agriculture Organization and World Health Organisation to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. Accordingly, the products used in VLCD should comply with the following composition and quality factors.[154] CODEX also defines that the content of the diet should be not less than 50 g of protein, 50 g of carbohydrate, 3 g of linoleic acid as fat and vitamins and minerals supplement.[154]

Patients may experience polyuria in first few weeks of VLCD/LCD because of loss of fluid and glycogen.[155] This effect is enhanced by a reduction in plasma insulin levels causing less sodium retention; increasing levels of natriuretic hormones and glucagon; increasing ketonuria causing extra sodium and potassium loss together with water. There are a number of minor side effects also reported with VLCD/LCD. Dry mouth, constipation, headache, dizziness/orthostatic hypotension, fatigue, cold intolerance, dry skin, menstrual irregularities and hair loss are common side effects during therapy. Occasionally, cholelithiasis has been reported although it is more frequently in United State compared to Europe. A certain amount of fat is required to stimulate gall bladder contraction and subsequent emptying of bile preventing stone formation. In Europe, it is

requirement that higher fatty acid of 7g or more per day is included in VLCD and this probably explains the difference in incidence. The National Institute of Health has therefore recommended consuming an extra 10g of fat.[156] Fatal dysrrhythmias were reported in 1970s when there was imbalance use of VLCD.[157, 158] Extensive evaluation of modern VLCD/LCD did not show increased risk of cardiac arrhythmia nor prolonged QT interval.[159]

VLCD/LCDs are used to promote short-term weight loss in obese patients. However, long-term maintenance of weight loss is generally poor. Anderson, J.W et al[160] followed up a group of patients who achieved an average 29.7kg weight loss induced by VLCD over 5 months. Subjects regained an average of 2.5% per month of their lost weight during the first two to three years of follow-up. An average of 73.4% of their weight loss was regained during the first three years. When successful weight maintenance was defined as maintaining a weight loss of 5% or 10% of initial (pre-treatment) body weight, 40% were maintaining a 5% weight loss at five years and 25% were maintaining a weight loss of 10% at 7 years.

Gilden and Wadden[161] conducted a meta-analysis of six randomised control trials comparing VLCD and LCD. In the short term, participants in the VLCD programme achieved better weight loss compared with LCD with the mean weight loss of 16.1±1.6% Vs 9.7 ±2.4% respectively (p <0.001). At follow up (mean =1.9± 1.6 years) assessment, the difference between these two groups were small (mean weight loss of 6.3 ± 3.2% in VLCD and 5 ± 4% and LCD) and not significant (p> 0.2). As regards to weight related co-morbidities, there was no difference in HbA$_{1C}$, lipid profile or systolic blood pressure. Changes in the cardiovascular risk factors were not measured. Side effects encounter included mild reversible alopecia, precipitation of gouty arthritis, transient cold intolerance and constipation were common in VLCD group. The duration of each therapy was not associated with the degree of weight loss.

Weight loss induced by LCD has some effects on the sympathetic nervous system. Sowers et al[162] demonstrated following VLCD, there was reduction in both blood

pressure (BP) and NE level at supine and upright. A significant correlation in reduction in NE level and BP was also observed. The authors concluded that the reduction in BP in obese patients on diet with VLCD seems to result in at least in part from reduced SNS activity.

Pharmacotherapy following VLCD provides better long term weight maintenance compared with placebo. Finer et al[163] conducted a randomised controlled study where 8 weeks of VLCD was followed by either dexfenfluramine 15mg bd or placebo for 26 weeks. With dexfenfluramine therapy, patients achieved a further 5.8 ± 1.8 kg weight loss in contrast to placebo group who regained 2.9 ± 1.3 kg. The total weight loss after 34 weeks was 21.3 ± 2.6 versus 11.3 ± 1.9 kg. Anderson et al also demonstrated similar findings.[163, 164] Dexfenfluramine was however removed from the market in 1997 because of its association with valvulopathy.[165] Similarly, Apfelbaum et al[166] showed that, after initial 4 weeks of a VLCD, during which patients lost 7.6 kg, those randomized to 1 year of treatment with sibutramine achieved a cumulative loss of 12.8 kg at the end of this time, compared with a loss of 7.1 kg for placebo-treated individuals (p=0.004). Mathus-Vliegen[167] prescribed a VLCD for 3 months, which induced an initial weight loss of 15.2 kg. Participants were then randomly assigned to sibutramine (10 mg/d) or placebo for an additional 15 months. At month 18, patients in the sibutramine group maintained a loss of 10.7 kg, compared with 8.5 kg for those prescribed placebo ($p = 0.008$). Thus, sibutramine slowed but did not prevent weight regain after a 15-kg loss.

1.5.2. Anti-obesity drugs

Pharmacotherapy for obesity and diabetes has been well advanced recently. Newer therapies have been developed targeting on different receptors as described in (Figure 1.7). Some of these drug groups are at an early phase of drug development. At the time this study took place, three drugs were available and licensed: orlistat, sibutramine and rimonabant. However, subsequently sibutramine and rimonabant were withdrawn.

Figure 1.7.   Drug targets for anti-obesity and anti-diabetes therapy

CCK = cholecystokinin, PYY= peptide YY, GLP-1 = Glucagon like peptide-1, DDPIV inhibitor = dipeptidyl peptidase-4 inhibitor, CARD= cocaine and amphetamine regulated transcript, NPY= neuropeptide Y, SGLT2 inhibitor =Sodium Glucose Cotransporter Inhibitors. (Figure was provided by the courtesy of Dr N Finer).
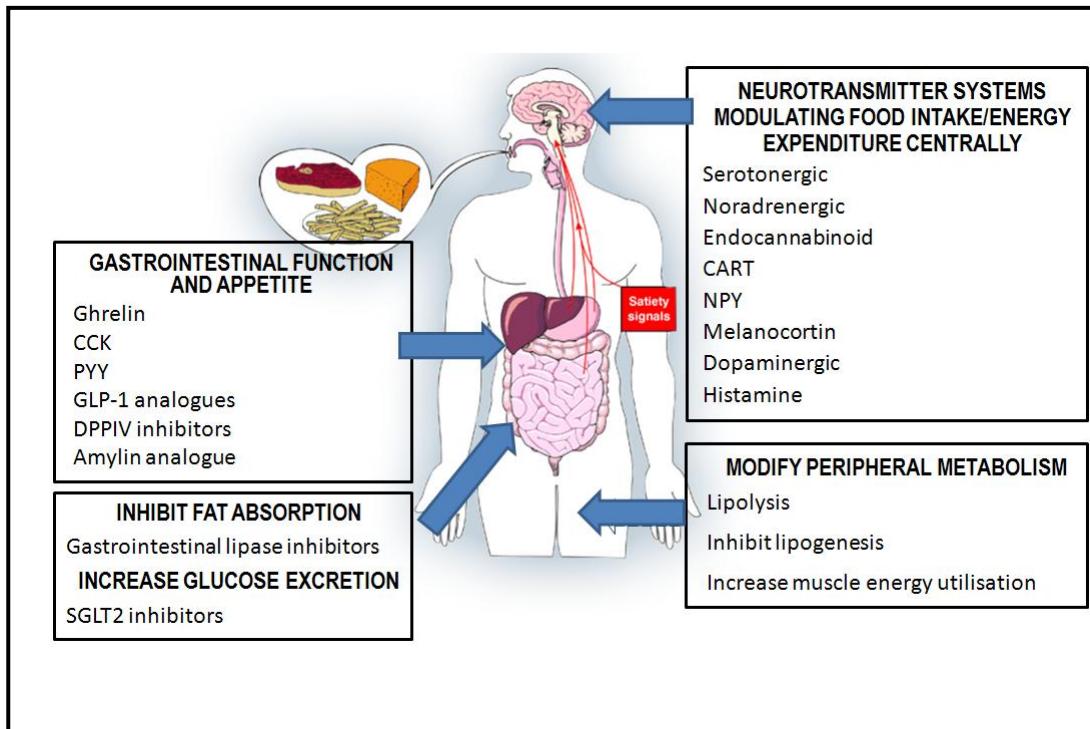
Orlistat is a potent, specific long acting gastrointestinal lipase inhibitor. It forms a covalent bond with the serine site of the gastric and pancreatic lipase. It prevents hydrolysis of dietary triglyceride into absorbable free fatty acid and monoglyceride and therefore limits absorption of these into the circulation.[168] It is indicated in conjunction with calorie restriction in obese patients with BMI $\geq$30kg/m^2 or overweight patients (BMI $\geq$28kg/m^2) with associated risk factors (hypertension, diabetes and hypercholesterolaemia). The 4 year XENDOS (Xenicle prevention of diabetes in obese subjects) clinical trial[169] demonstrated that 60% patients on orlistat and 35% patients on placebo lost > 5% of initial body weight at the end of 12 weeks. Of those, 63% and 52% of orlistat and placebo group respectively continued to lose >10% at the end of one year.

Rimonabant was a cannabinoid receptor 1 (CB$_1$ receptor) antagonist. CB$_1$ receptors have been shown to play an important role in food intake, energy balance and are directly implicated in lipid and glucose metabolism. CB$_1$ receptors are located centrally in the hypothalamus[170] and mesolimbic[171] region in the brain, and peripherally in adipose tissue, liver, skeletal muscle and the gastro-intestinal tract. In the brain, the hypothalamus plays a principal role in the control of feeding and regulation of body weight, and CB$_1$ receptor stimulation leads to dopamine release in the nucleus accumbens shell, which increases motivation to eat.[172] These effects result in increased food intake and fat accumulation. Peripherally, endocannabinoid system (ECS) overactivation promotes lipogenesis at the level of adipose tissue [173] and the liver.[174] ECS activity in the GI tract interferes with feelings of satiety, and CB$_1$ receptor stimulation of skeletal muscle decreases glucose uptake. All of these central and peripheral effects contribute to the increased risk of dyslipidaemia, insulin resistance, glucose intolerance and increased cardiometabolic risk.[172] Blocking CB$_1$ receptors by Rimonanbant, therefore produced weight loss. In the RIO- North America (rimonabant in obesity) study, patients on rimonabant 20mg for one year achieved weight losses of ≥5% and ≥10% in over 62% and 32% of patients respectively, compared with 33% and 16% respectively, of control subjects on placebos.[175]

However, there were concerns regarding major psychiatric symptoms in patients taking Rimonabant. Up to the end of Jan 2008, there was1971 reported reactions from UK. 44% (876) of those were psychiatric reactions. The most common psychiatric reactions were depression and related disorders of mood and associated symptoms. 52 reactions involved suicidal and self-harming thoughts or behaviours, most of which were suicidal ideations (42 reports). Because of its major side effect of psychiatric symptoms, the Medicines and Health-care-products Regulatory Agency (MHRA) alerted health care professional in May 2008.[176] The European Medicines Agency (EMEA) recommended the suspension of the marketing authorisation for Rimonabant in October 2008. In December 2008, the drug was withdrawn when the marketing authorisation holder

(MAH) responsible it (Sanofi-Aventis) notified the European Commission of its decision to voluntarily withdraw its marketing authorisation.

Sibutramine was a beta phenethylamine which selectively inhibited the reuptake of serotonin and norepinephrine and to a lesser degree, dopamine (Figure 1.8). It produced weight loss mainly by enhancing satiety[177] and to some extent increasing thermogenesis.[178, 179] In a meta analysis of sibutramine studies,[180] a statistically significant beneficial weight loss effect of sibutramine therapy was noted compared to placebo (a 4.3 kg with 95% CI: 3.6-4.9 kg versus 4.6% with 95% CI: 3.8-5.4%) greater weight loss. In addition, 15% (95% CI: 4-27%) more subjects achieved a 10% or greater weight loss with sibutramine than with placebo therapy.

Figure 1. 8.   Mechanism of Sibutramine demonstrating the inhibition of norepinephrine and serotonin reuptake from nerve endings

Pictures adapted from:
http://www.drugabuse.gov/pubs/teaching/teaching4/Teaching.html
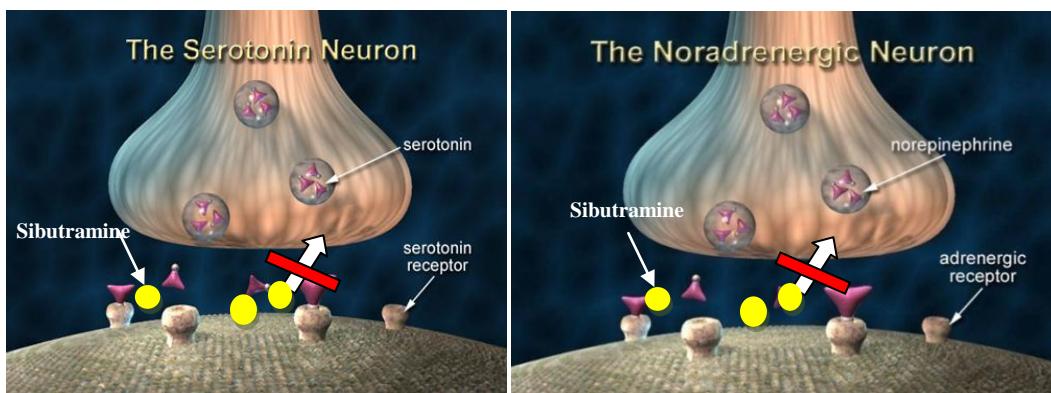
In Jan 2010, European Medicines Agency (EMA) issue a "dear health care professional letter" following a review the data from a large study "The Sibutramine Cardiovascular outcomes (SCOUT) study".[181] It was a randomised, double-blind, placebo controlled study in approximately 10,000 obese and overweight patients with cardiovascular disease and/or type 2 diabetes treated over a six year period. The purpose of the study

was to determine the long-term effect of sibutramine treatment on cardiovascular outcomes in overweight and obese patients at risk of a cardiovascular event. There was a 16% increased risk of cardiovascular events (myocardial infarction and stroke) in sibutramine treated patient compared with placebo (hazard ratio 1.161 [95% CI 1.029–1.311]; p=0.016). The Committee noted that the mean weight loss with sibutramine was approximately 2-4 kg more than placebo in most trials. It concluded that the risk of the medicine outweighed the benefit and therefore recommended the suspension of marketing authorisation across Europe.

CHAPTER 2:

HYPOTHESIS

CHAPTER 2:   HYPOTHESIS

2.1.   Study hypothesis

The hypothesis of this study was that weight loss intervention in obese patients with heart failure or high CVD risk would improve cardiac performance measured as peak VO_2 and reduce biomarkers of cardiac risks.

2.2.   Study Objectives

The primary objective of this study was to investigate the effect of acute weight loss (6 weeks) and weight loss maintenance (16 weeks) on cardiac reserve in obese patients with heart failure or high cardiovascular risk.

The secondary objectives were

- to describe measures of cardiac function and risks (plasma BNP, resting plasma norepinephrine, autonomic activities, cardiac output, left ventricular function and antioxidant profile) in obese patients with heart failure and/or high CVD risk.
- to compare two methodologies of body composition measurement i.e., Four compartment model and quantitative MRI.

2.3.   End Points

The primary end point of the study was peak VO_2 following acute weight loss (week 6) and weight loss maintenance phase (week16).

The secondary end points were

- LVEF, HRV, resting plasma BNP, resting plasma norepinephrine, antioxidant profile, ALT, cholesterol, hsCRP, fasting plasma glucose and insulin, adiponectin, leptin, TNF-α and IL-6, exploratory analysis relating changing in the endpoints to FM and FFM changes.
- Comparison of FM measured by 4C model *Vs* quantitative MRI.

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CHAPTER 3:

METHODS

CHAPTER 3:   METHODS

3.1.   Study design

The original plan of this study was to conduct a randomised controlled study in obese patients with heart failure, comparing a weight loss intervention with intensive diet Vs non-intervention. A non-intervention group was assumed to be necessary to provide information on the background progression or regression of heart failure without weight loss and in the impossibility of providing a placebo weight loss diet. Since the study is designed to assess the benefit (or harm) of weight loss, it would be ethically appropriate for such a non-intervention control group. However, since it seemed unlikely that there will be significantly measurable changes in cardiac function in the non-intervention group over the study period of 16 weeks, randomisation would be 3:1 intervention/ non-intervention.

3.1.1.   Sample size calculation

G*Power 3.1 software (Heinrich-Heinec University, Duesseldorf, Geremy) was used for the power calculations and were based on a study by Kanoupakis et al.[182] This was a study of patients undergoing bariatric surgery with a mean BMI of 52 kg/m^2, they showed a peak VO_2 at baseline of 19 ml/kg/min ±3.9 (SD) that improved by 40% after a 64% weight loss. The study was on metabolically healthy more obese individuals who lost more weight than our study population. We therefore adjusted our assumptions as Table 3.1. Different sample sizes were then examined and plotted against power (Figure 3.1). It was calculated that 23 subjects would be needed to detect a 10% improvement in peak VO_2 after a 10% weight loss at an effect size of 0.655, p value <0.05 and 85% power (Table 3.2). To achieve 3:1 intervention Vs non-intervention ratio, the number in intervention group was increased to 24 with an additional 8 patients were to be recruited as the non-intervention control group.

Table 3. 1.   Assumptions of our study comparing with Kanoupakis et al for sample size calculation

Parameter	Kanoupakis et al	Our study
Baseline weight	139kg	110kg
Percentage weigh loss	64%	10%
Peak VO_2 at baseline	19%	19%
Improvement in peak VO_2 at the end of intervention	40%	10%
SD difference	3.9%	2.9%[*]
Power		85%

*SD was estimated at 75% of Kanoupakis study in a view of smaller changes in weight and peak VO_2.

Figure 3. 1.   X-Y plot for a range of sample size against power
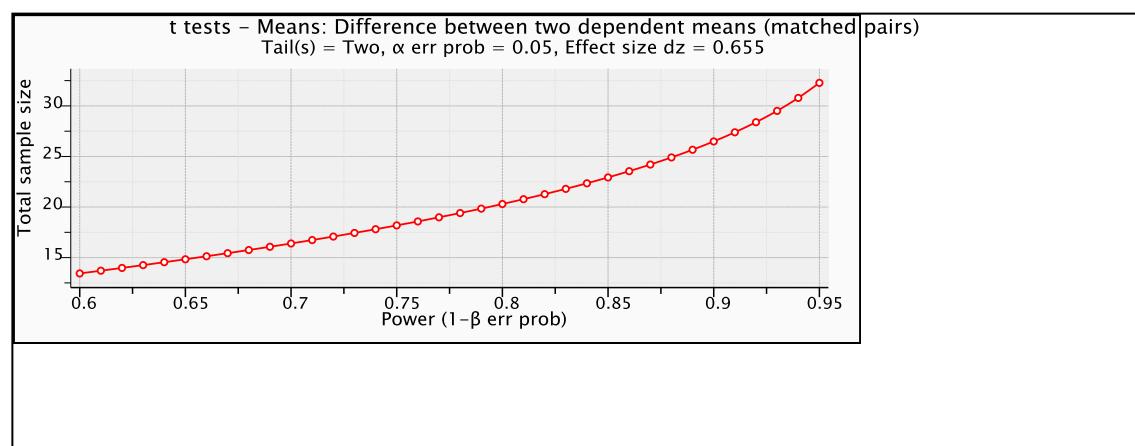
Table 3.2.   Sample size calculation generated by G*Power 3.1 software

t tests - Means: Difference between two dependent means (matched pairs)		
Analysis:	A priori: Compute required sample size	
Input:	Tail(s)	= Two
	Effect size dz	= 0.6551724
	α err prob	= 0.05
	Power (1-β err prob)	= 0.85
Output:	Noncentrality parameter δ	= 3.1420964
	Critical t	= 2.0738731
	Df	= 22
	Total sample size	= 23
	Actual power	= 0.8513855

Randomisation was carried out using the web base tool "Research Randomizer" at
http://www.randomizer.org. 9 set of 4 unique numbers per set was calculated with the
range from 1 to 4. Number 1, 2 and 3 were allocated for active weight management and
number 4 was allocated for control. The randomisation sheet was kept in a research
folder at the Clinical research facility and study nurse was responsible for identifing the
code at the end of the screening visit.

A pilot study of 6 subjects was undertaken in advance of the full study to assess
feasibility and safety. Due to recruitment and other logistical issues, this thesis is based
upon the pilot study of 6 patients and a further 8 patients from the main study (all of
whom had the weight loss intervention) of whom 11 completed the trial.

The study was approved by South Cambridgeshire Ethics committees on the 26 April
2006. (REC ref: 06/Q0108/37) and sponsored by Research and Development
Department, Cambridge University Hospital NHS Foundation Trust. (R&D ref:
A090622).

3.2. Study population

Eleven obese patients with heart failure or additional cardiovascular risk were recruited for study. Obesity was defined as a BMI of $\geq$30 kg/m^2.

3.2.1. Inclusion criteria

Subjects met all inclusion criteria.

- Obese patients defined at BMI of $\geq$30 and $\leq$ 50 kg/m^2

- Age $\geq$25 and $\leq$ 70 years

- Stable heart failure patients of NHYA class II or III OR subjects with one of additional cardiovascular risks

- Hypertension
- Dyslipidaemia defined by having lipid lowering therapy or HDL<1 mmol/l, LDL >3.5 mmol/l or total cholesterol and HDL ratio >5
- Diabetes or pre-diabetes (impaired fasting glucose level of $\geq$ 6.1 mmol/l)
- History of ischaemic heart disease

The diagnosis of heart failure was based on standard criteria of symptoms of more than 6 months with confirmed LV impairment on echocardiogram.

3.2.2. Exclusion criteria

- Those unable to give informed consent

- Recent history weight loss (2 kg or 5% weight loss within 3 months) prior to study

- Patients who had changed their heart failure medication in the previous 6 weeks or who were likely to have therapy changes during the study period (16 weeks)

- Subjects with renal impairment defined by creatinine >170 µmol/l

- Obese subjects who were unlikely or unwilling to the adhere to weight loss diet

- Subjects who are unable to do the cardiopulmonary exercise

- Peripheral vascular disease of a degree to prohibit undertaking exercise test

- Recent (within three months) history of acute myocardial infarction or unstable angina

- Uncontrolled arrhythmias causing symptoms or haemodynamic compromise (systolic BP <90 mmHg)

- Any significant valvular heart disease

- Inadequately controlled hypertension defined by resting blood pressure > 170/95mmHg

- Pregnancy (actual or planned)

3.3.   Study Schedule

3.3.1. Recruitment

Obese patients with heart failure or additional cardiovascular risk were recruited from the cardiology, diabetes, hypertension and obesity clinics at Addenbrooke's hospital, Cambridge. Recruitment was extended to General practitioners, the community heart failure nurse and other cardiology departments at Luton and Dunstable, Bedford and West Suffolk Hospitals. With the permission from research and development department (R&D) at Addenbrooke's Hospital discharge summary database was also search for the codes of the obesity (E66) and heart failure (I50) in the diagnosis and or co-morbidities for previous one year for all in patient admissions. Echocardiogram reports were also reviewed for patients with heart failure through out the study. Detail of the recruitment process was illustrated on Figure 3.2.

Figure 3. 2.   Recruitment Process

72 Potential subjects identified

- 8 -Diabetes Clinic
- 4 -Obesity Clinic
- 4 -Hypertension Clinic
- 5 -Cardiology Clinic
- 2 -General Practitioners
- 2 -Regional cardiology departments
- 24 -Hospital discharge letters
- 23 -Echocardiography reports

- 5 subjects identified from Hospital discharge letters died
- 14 subjects of above list were not eligible
- 19 subjects from Echo report list were not eligible

34 Subjects invited to study
- 19 Face to face invitations
- 15 Invitations by letters

17 Subjects accepted invitation
- 12 from face to face invitation
- 5 from invitation by letter

2 Subjects failed at screening (1- not able to exercise, 1- severe LVEF <15% and would not be safe to take part in the study)

15 subjects recruited for the study
- 6 pilots
- 9 subjects for main study (first 3 were randomised)

2 subjects withdrew from study
- 1 - could not tolerate LELD
- 1 – diagnosed with breast cancer

12 subjects completed the study
- 4 subject from pilot study
- 8 subjects for main study (first 3 were randomised- 1 control and 2 LELD group)

Data from 1 controlled subject was excluded from analysis

Complete data was presented on 11 subjects

Subjects were given information leaflets about the study so that they could read and discuss them at home prior to giving consent at a later date (>48 hours). Patients then attended one screening and 3 main measurement visits (week 0, 6 and 16). In addition, they attended 10 interim visits for monitoring and safety assessments. (Figure 3.3)

Figure 3. 3.   Schematic diagram of schedules for each subject

(LELD = low energy liquid diet, WMD = weight loss maintenance diet, P= pre-screening (invitation), S= screening visit, M= Measurement visit, I = interim visits)
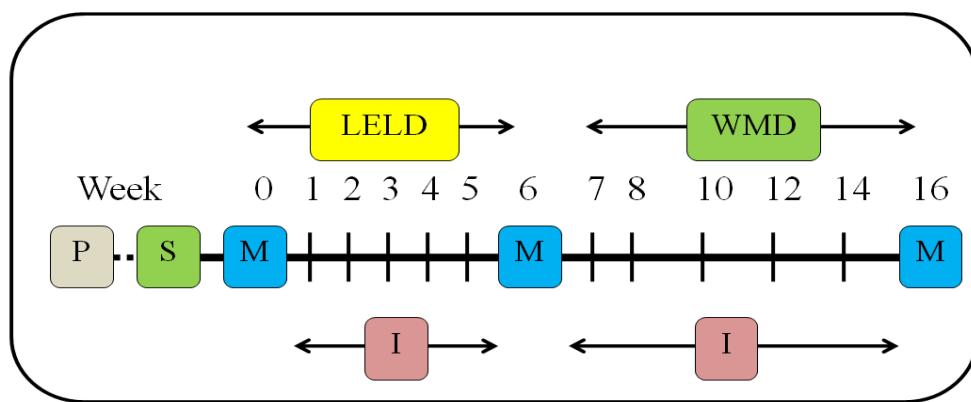
3.3.2. Screening visit

A detailed medical history and physical examination was undertaken to assess whether subjects were eligible for the study. Subjects fulfilling the entry criteria of the study were asked to give informed consent. Blood for biochemistry and antioxidant profile was taken. Patients underwent cardiopulmonary exercise testing for peak VO_2 in order to acclimatise themselves with the equipment and the procedure. An echocardiogram was performed for confirmation of the diagnosis of heart failure as well as exclusion of any significant valvular heart disease. Following this visit, patients who still met the inclusion criteria were invited to continue in the study.

3.3.3. Measurement Visits (week 0, 6 and 16) and interim visits

Subjects attended the Clinical Research Facility (CRF) at Addenbrooke's Centre of Clinical Investigation (ACCI) at 9am on the study day. Subjects stayed overnight (i.e., for 36 hours) and all measurements listed were performed during the stay in the department.

3.4. Induction of acute weight loss and weight loss maintenance

A milk-based low energy liquid diet was provided for induction of acute weight loss. The terminology of low energy liquid diet (LELD) was used because it is low in energy (800- 1000kcal) and liquid. Both factors are important components of the intervention. The regimen is routinely used to achieve rapid weight loss by specialists in weight loss management clinics worldwide and also in the Obesity Clinic at Addenbrooke's Hospital. We prescribed a diet based on semi skimmed milk to provide between 800 kcals/day for women with BMI <40kg/m^2 and 1000 calories per day for all men and women with a higher BMI. Patients were instructed to consume $2 - 2.5$ gram of sodium intake in the form of either Bovril or stock cubes. In addition, one to two sachet of fybogel ('Ispaghula husk') and mineral and vitamin supplements (two Sanatogen gold® tablets- Bayer plc, Berkshire, UK) were also provided everyday. Each Sanatogen gold® tablet includes Vitamin A 800ug, Vitamin D 5ug, Vitamin E 10mg, Vitamin C 60mg, Thiamin 1.4mg, Riboflavin 1.6mg, Niacin 18mg, Vitamin B6 2mg, Folic Acid 200ug, Vitamin B12 1ug, Biotin 0.15mg, Pantothenic Acid 6mg, Beta Carotene 400ug, Vitamin K 30ug, Calcium 173mg, Phosphorus 144mg, Iron 14mg, Magnesium 120mg, Zinc 15mg, Iodine 150ug, Copper 2mg, Boron 150ug, Chloride 36.3mg, Chromium 25ug, Manganese 2.5mg, Molybdenum 25ug, Nickel 5ug, Potassium 40mg , Selenium 25ug, Silicon 2mg mg, Tin 10ug and Vanadium 10ug). This was the sole source of nutrition for the first 6 weeks. For those who found 'simple' milk unpalatable, we provided an alternative diet using commercially available products (e.g., Slim Fast®, Surrey, UK). Patients maintained the liquid diet for 6 weeks during which time they were seen weekly. After 6 weeks, solid food was reintroduced into their diet, based upon an energy intake that would be weight loss maintaining (WLM). The energy value of the WLM

diet was calculated to match the daily energy expenditure. It was derived from standard equations relating height, weight and age to basal metabolic rate (BMR) and Harris Benedict Formula[183] as follow:

Equation 2

For men,

BMR = 66.5 + (13.75 x weight in kg) + (5.003 x height in cm) - (6.775 x age)

For women,

BMR = 655.1 + (9.563 x weight in kg) + (1.850 x height cm) - (4.676 x age)

Total Caloric Requirements equal the BMR multiplied by the activity factors. Activity factors range from 1.2 to over 2 (Table 3.3).

Table 3. 3.   Harris Benedict Formula for activity levels/factors

Activity level	Description of activity	Activity Factor: (BMR to be multiply by)
sedentary	little or no exercise	1.2
lightly active	light exercise/sports 1-3 days/week	1.375
moderately active	moderate exercise/sports 3-5 days/week	1.55
very active	hard exercise/sports 6-7 days a week	1.725
extra active	very hard exercise/sports & physical job or 2x training	1.9

In order to allow for incomplete compliance, no adjustment for energy costs of activity or exercise were made, so in practice this diet only gave 80% approximately of energy needs (based upon a predicted physical activity level of 1.2). Orlistat (Roche Xenical®,

Welwyn Garden City, UK) 120mg tds was used in order to aid weight loss maintenance during this second phase.

3.5. Cardiopulmonary exercise testing

3.5.1. Introduction

Cardiopulmonary exercise testing (CPET) is relatively non-invasive, dynamic, physiological test that allows the simultaneous study of responses from cardiovascular and ventilatory systems to a known exercise stress (both sub-maximal and peak exercise) through the measurement of gas exchange at the airway: oxygen uptake (VO_2), carbon dioxide output (VCO_2), and minute ventilation (V_E), and the cardiovascular measurements: ECG, heart rate, and blood pressure. These cardiovascular measurements, importantly, interrelate with the gas exchange measurement. This interrelation provides additional information because it relates them to the actual energy expended during exercise rather than relying on indirect estimates of energy. VO_2 and VCO_2 reflect oxygen consumption and carbon dioxide production during cellular metabolism. Wasserman[184] described the chain of events in gas exchanges during exercise (Figure 3.4). The increase in ventilation during exercise causes recruitment and vasodilatation of the pulmonary blood vessels, increase pulmonary blood flow, and subsequent increase in cardiac output (stroke volume and heart rate) and the dilatation of the selected peripheral vascular bed. This in turn causes a large increase in oxygen extraction and utilization by the muscle (QO_2), resulting in an increased CO_2 production (QCO_2) which is then transported to the lungs via the systemic and pulmonary circulation. Therefore, gas exchange measurements reflect functions of not only pulmonary but also cardiac and vascular function. The use of CPET in the management of various cardiopulmonary diseases is increasing with the understanding that resting pulmonary and cardiac function testing cannot reliably predict exercise performance and functional capacity. Furthermore, overall health status correlates better with exercise tolerance rather than with resting measurements. It provides relevant information for clinical decision making e.g., assessment for heart transplantation.[110]

Figure 3. 4. Wasserman's Wheels showing gas exchanges during exercise
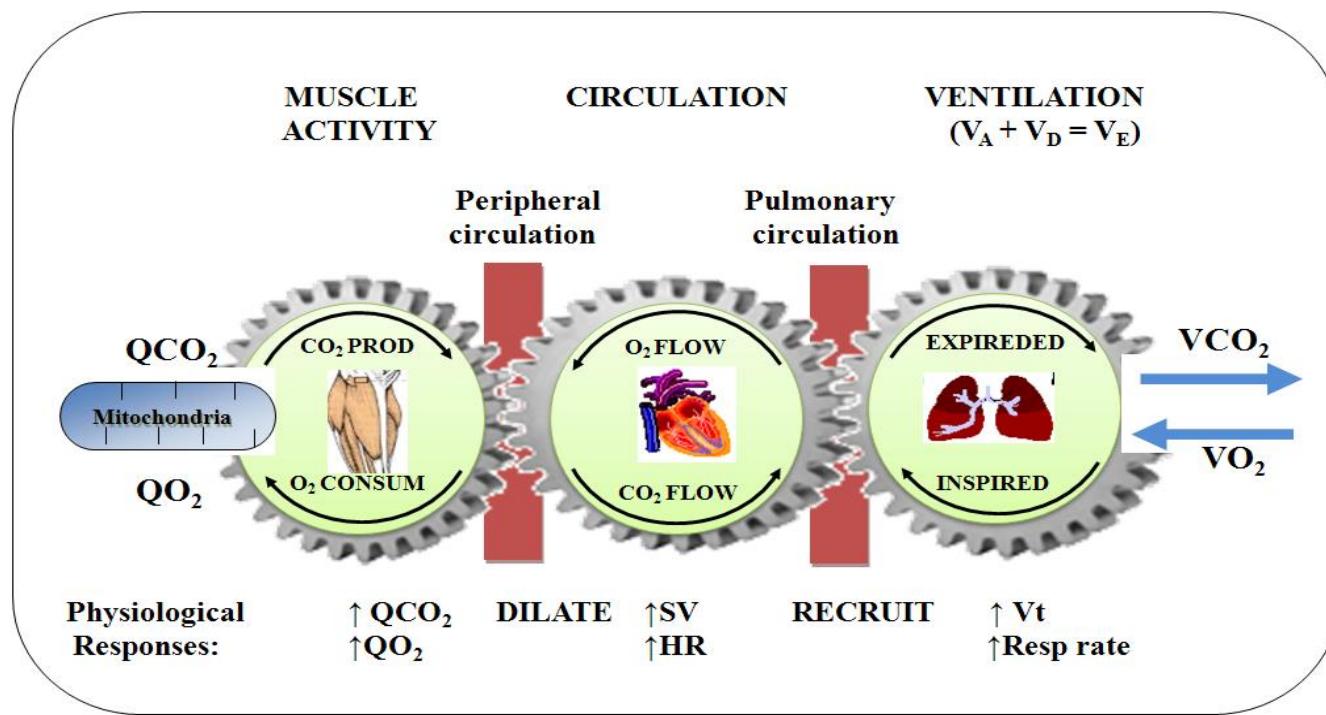
Resp rate = respiratory rate, Vt= tital volume (volume of air inspired with each breath), V_A = alveolar ventilation (total volume of fresh air entering the alveoli per minute), V_D = physiological dead space, SV =stroke volume, HR = heart rate, QCO_2 = cellular carbon dioxide output, QO_2 = cellular oxygen uptake, CO_2 PROD = carbon dioxide production, O_2 CONSUM = oxygen consumption. The functional interdependence of the physiological parameters was represented by gears. (adapted from reference [184])

3.5.2. Exercise equipments

3.5.2.1.   MedGraphics Ultima^{TM} Cardio2 system

This is a combined system of Breezesuite Gas Exchange software and PC based ECG (Medical Graphics Corporation, Saint Paul, Minnesota.). It provides an integrated 12-lead stress ECG combined with breath-by- breath measurement of O_2 and CO_2 production in the air. Air flow calibration was performed each day of the CPET and gas analyser calibration was performed prior to each test according to manufacturer protocol.

3.5.2.2.   The motor-driven treadmill

It imposes progressively increasing exercise stress through a combination of speed and grade (elevation) increases.

3.5.2.3.   Blood pressure machine

A semi-automated recorder (Quinton STBP-680; Quinton Instruments, Seattle, Wash) was used to measure blood pressure non-invasively every two minutes throughout the test.

3.5.3. Procedure

a.   The subject was interviewed again to make sure there was no contraindication for CPET.

b.   The detail procedure was explained with an emphasis to report any chest pain, breathlessness, dizziness, fainting or should they feel they needed to stop immediately. An Emergency stop button was provided should he/she needed to stop immediately.

c.   At least two staff (myself and one staff nurse/ technician) were present throughout the study.

d.   A 12-lead ECG, blood pressure and oxygen saturation monitoring equipments were attached to the patient and baseline measurements were performed. ECG and oxygen saturation were recorded continuously throughout the study. Blood pressure was recorded every two minutes.

e.   The appropriate preVent mask (Medical Graphics Corporation, Saint Paul, Minnesota.) was chosen and fitted according to the instruction to make sure there was no air leak (Figure 3.5) and it was then attached to the pneumotach which is connected to the gas analyser.

f.   CPET started according to the Naughton protocol[185] (Figure 3.6). Patients were encouraged to exercise to maximum effort guided by perceived effort of exertion according to Borg's scale (Table 3.5) and anaerobic threshold (Figure 3.7).

g.   The aim was to achieve Borg's scale of 17.

h.   Exercise was discontinued if the patients expressed any symptom of chest pain or distress or met any of the termination criteria (Table 3.4).

3.5.4. preVent Mask Selection and Fitting Instructions:

Correct selection and fitting of the mask was done to ensure a leak-free fit for both exercise and metabolism assessment (Figure 3.5). A proper fit was performed as follows:

a.   The narrow strap (upper) was positioned on the back, near the "crown" of the head and the wide strap (lower) was positioned across the back of the neck.

b.   The top of the mask with the shapeable aluminium strap was pulled up to the top (bridge) of the nose and formed to fit securely around the nose.

c.   The bottom of the mask was pulled securely under the chin so that the material between the nose and chin was slightly stretched. The mask wrapped under the chin about 2.5cm.

d.   The sensor coupler was located comfortably in front of the mouth.

Figure 3. 5. **photograph showing fitting instructions of preVent Mask**
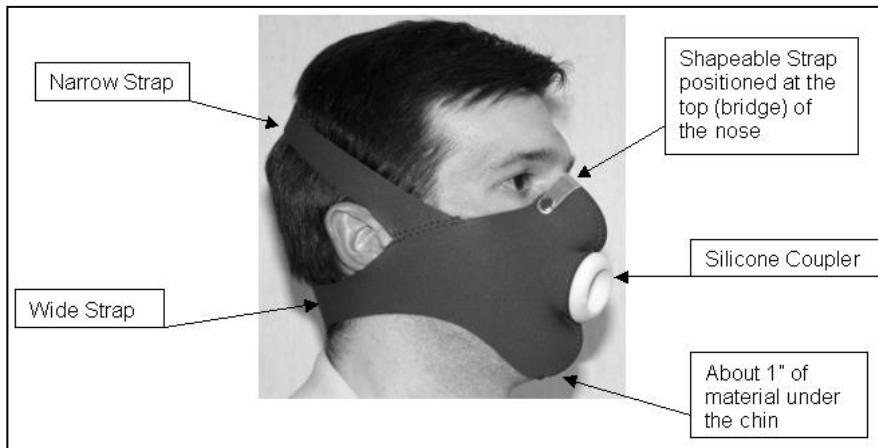
3.5.5. Naughton Exercise Protocol

The modified Naughton protocol (Figure 3.6) starts at a lower metabolic equivalence (MET) than full Bruce protocol. Workload increased by 1-MET per stage, thus allowing better-tolerated gradual progression in exercise and a more accurate assessment of exertional capacity. There are seven stages with each stage lasting two minutes. During the first stage of exercise, the treadmill speed was set to 1mph and 0% grade. For stage 2 (minute three to four), the speed was increased to 2.0 mph while the grade remained at 0%. From stage 3 onward (minutes 5), the speed was maintained at 2.0 mph, and the grade was increased 3.5% every two min until it reached the stage 7 (minute 12) and then no more increase in exercise work load but continue the test to patient's maximum effort. A set of strict criteria for the termination of exercise was followed (Table 3.4).[116] Patients were encouraged to exercise to their maximal effort. It was guided by Borg's scale of perceived exertion (Table 3.5).

Figure 3. 6.   Schematic diagram of Naughton Exercise protocol
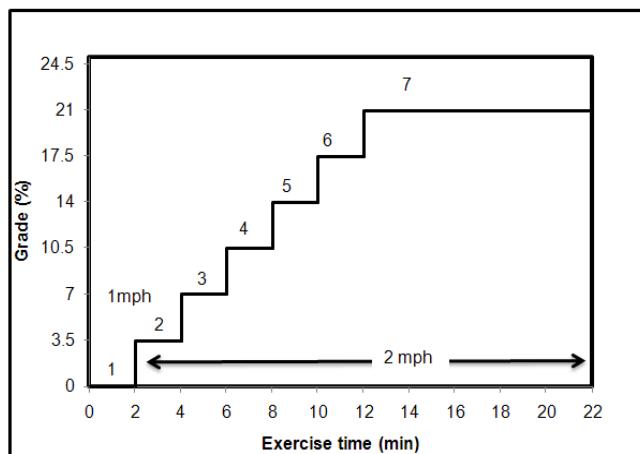
Table 3. 4   Indication for Exercise Termination

Chest pain suggestive of ischaemia

Ischaemic ECG changes

Complex ectopy

Second or third degree heart block

Fall in systolic pressure >20 mmHg from the highest value during the test

Hypertension (> 250mm Hg systolic; >120 mm Hg diastolic)

Severe de-saturation: $SaO_2 \leq 80\%$ when accompanied by symptoms and signs of severe hypoxemia

Sudden pallor

Loss of coordination

Mental confusion

Dizziness or faintness

Sign of respiratory failure

Table 3. 5.   Rate of Perceived Exertion: Borg's scale (6-20)

6	
7	very, very light
8	
9	very light
10	
11	fairly light
12	
13	somewhat hard
14	
15	hard
16	
17	very hard
18	
19	very, very hard
20	

3.5.6. Physiological measurements and definitions

- Peak VO_2

Peak VO_2 is the highest VO_2 during maximum exercise. It was referenced as absolute value (L/min) or corrected by individual's body mass (ml/kg/min).

- Respiratory exchange ratio (RER)

RER is also known as gas exchange ratio and is the ratio of VCO_2/VO_2.

- V-slope

It is the relationship of VCO_2 to VO_2.

- Anaerobic threshold (AT)

Anaerobic threshold (AT) is also called as lactate threshold and it indicates the point at which anaerobic metabolism is necessary to supplement the existing aerobic metabolism. It is the VO_2 at which changes in V-slope occurs. There are various ways to guide the point where AT occurs (Figure 3.7 and 3.8).

Figure 3. 7.   Physiological measurements during CPET
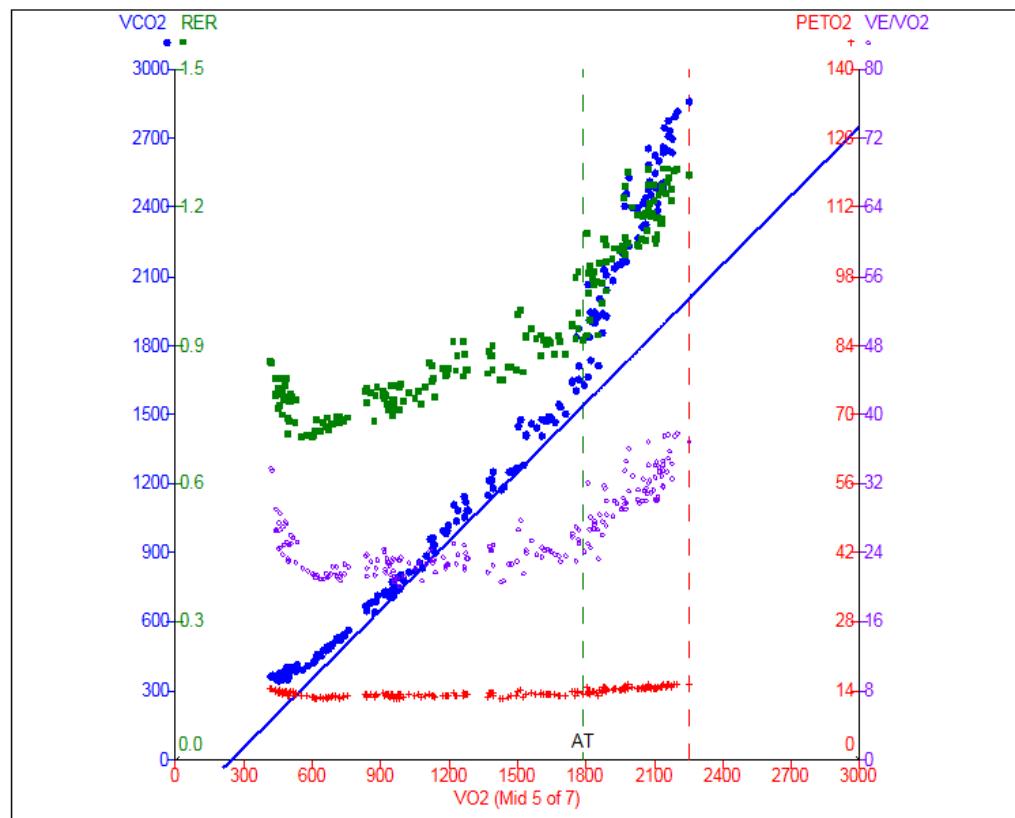
Example diagram generated by the software at the time of CPET.

VCO_2 = carbondioxide production, RER (green dot) = respiratory exchange ratio, AT(green dotted line)= anaerobic threshold, VO_2 = oxygen consumption, P_{ETO2}= end-tidal PO_2, VE= minute ventilation . At AT (green intermittent line), VCO_2 (blue dot) rises in proportionate to VO_2 and RER (green dot) starts to rise abruptly.

Figure 3. 8.   Schematic diagram showing determination of anaerobic threshold
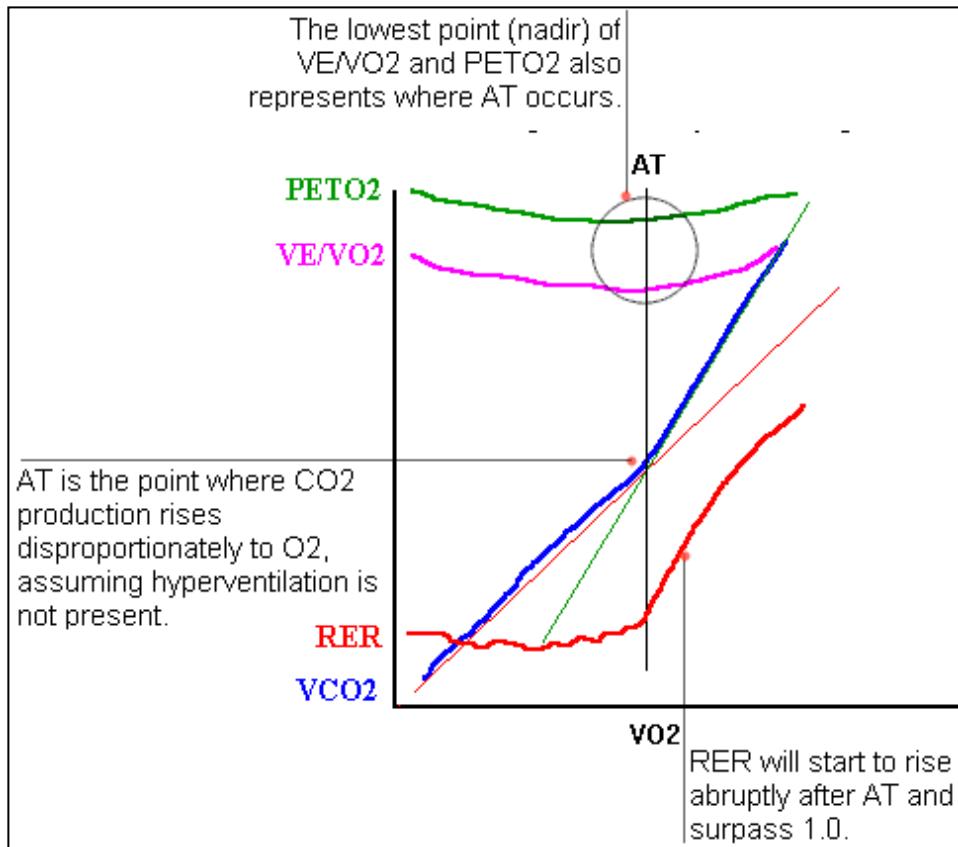
- **VE/ VCO$_2$ slope**

VE/ VCO$_2$ slope is the relationship between ventilation and the CO$_2$ production. (section 1.3.3). It represents the ventilatory response. The slope can be calculated from data recorded from initial exercise up to the time of either the peak exercise or the ventilatory threshold (VT). Both preVT and peak VE/VCO$_2$ slopes are significant predictors of cardiac mortality.[186] In our study, the data calculation was made only up to the point of peak exercise because some patients did not achieve ventilator threshold. Each VE and VCO$_2$ data, from the initiation of exercise to peak, were input into spreadsheet software (Microsoft Excel, Microsoft Corp., Bellevue, Washington) and the VE/VCO$_2$ slopes were then calculated via least squares linear regression (y = mx + b, m = slope, b= intersect) (Figure 3.9). Because both units of VE and VCO$_2$ were L/min, the slope did not have a unit.

55

Figure 3. 9.   Schematic diagram demonstrating VE/VCO$_2$ slope
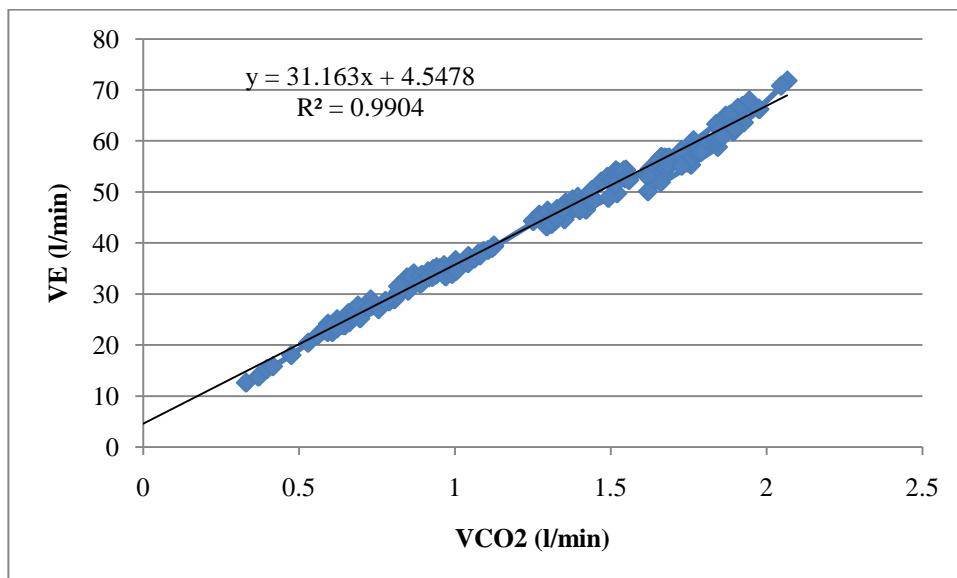
$VCO_2 = CO_2$ production, VE=minute ventilation, $y = mx + b$, m = slope, b= intersect

VE/VCO$_2$ slope demonstrating a linear relation between ventilation (VE) and the CO$_2$ production.

3.6.   Trans-thoracic Echocardiogram

Two dimensional echocardiograms were kindly performed by Dr Patrick Heck, Research Fellow in the cardiology department at each measurement visit by using Vivid 7 GE Medical Systems, Piscataway, New Jersey, USA. The operator variability was minimised as it was performed by the same individual. Data were captured at the time of each measurement. Analysis was performed offline by using the software Pac PC version 7 GE Medical Systems, USA. The contractile function of the ventricles was derived from quantitative measurement of the volume, area, and linear dimension of the left ventricular cavity. These measurements were obtained from multiple tomographic planes including parasternal long axis, parasternal short axis, apical four chamber, apical two chamber and apical long axis view.

The following measurements were performed.

- LVEF

- LV mass

- Systolic pulmonary arterial pressure

- End-diastolic thickness of the intra ventricular septum and posterior wall

- Right ventricular systolic pressure

The ventricular volume measured by 2D echocardiogram is based on tracing of the echocardiographic border at end-diastole and end-systole in one or more tomographic planes (Figure 3.10). This allows measurement of end-diastolic (EDV) and end-systolic volume (ESV) respectively which is required for ejection fraction determination. The stroke volume is the difference between EDV and ESV and subsequent ejection fraction can be calculated from the equation (Equation 3).

Equation 3   EF (%) = (SV / EDV) X 100%

EF = ejection fraction, SV = stroke volume, EDV= end diastolic volume

Figure 3. 10. Example for left ventricular volume calculation

Images on the left demonstrate the 2D echocardiographic view and those on the right demonstrates geographic model. For biplane apical method, apical four-chamber view and two-chamber view are used to trace the endocardial borders. This allowed the measurement of series of orthogonal diameters (a and b). The volume was then calculated based on the stacked disks assumption of "Simpson's rule" following the guidelines of Schiller and colleagues.[187] The single plane Ellipsoid method again used the apical 4 chamber view to calculate the 2D area (A) and length (L) in one single view. Hemisphere-cylinder method uses endocardial area at midventricular level (Am) on short axis view and length (L) in long axis view.
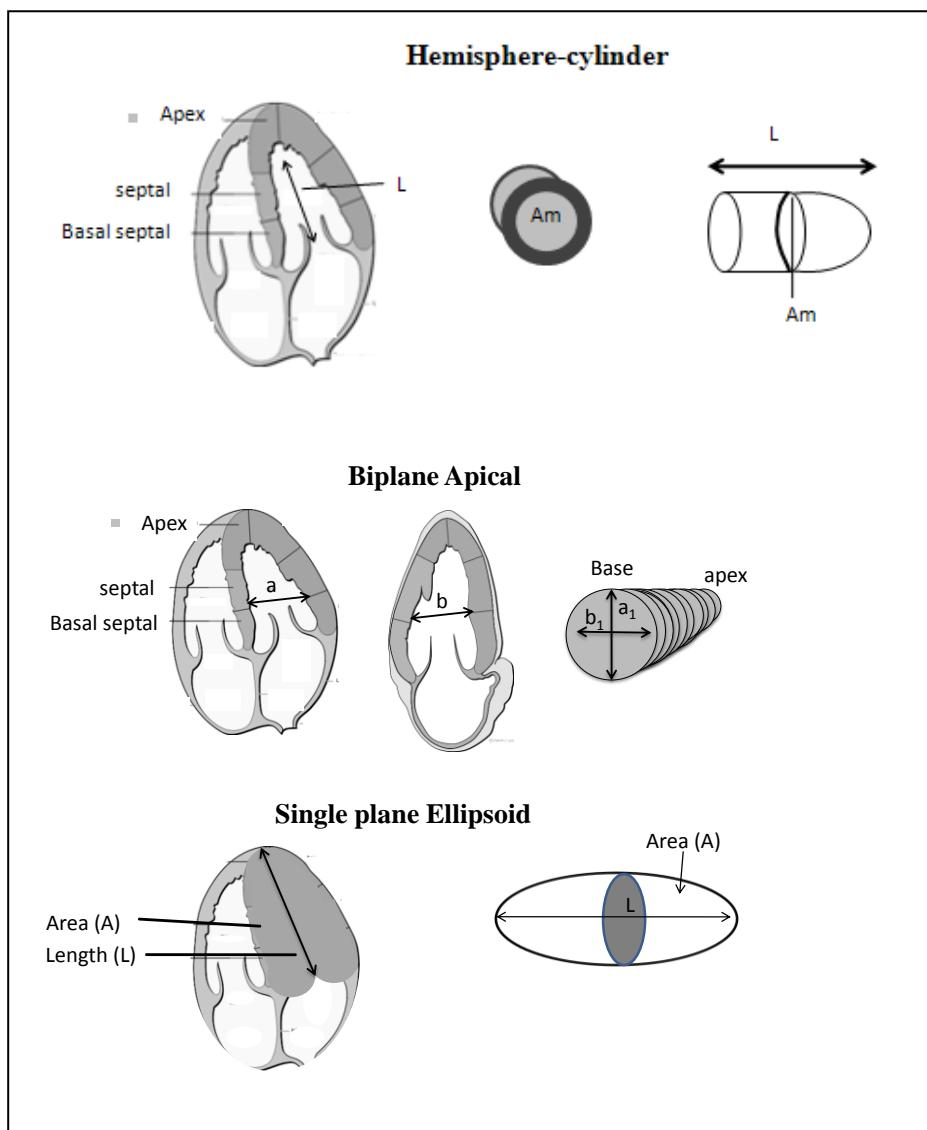
3.7. Heart rate variability

In normal cardiovascular physiology, it has been shown that heart rate and blood pressure spontaneously fluctuate.[188] Heart rate variability (HRV) is the temporal beat-to-beat variation/alteration of heart rate.[189] In a 12 lead ECG, the maximal positive QRS deflection is called "R" wave and duration between two adjacent "R" indicates R-R interval. In healthy subjects at resting condition, the R-R interval varies periodically with respiration; shortens during inspiration and lengthen during expiration. This phenomenon is known as sinus arrhythmia. This is predominantly mediated by post-synaptic parasympathetic (vagal) activity on the heart. Vagal withdrawal results in over-activation of counter regulatory system, the sympathetic control of cardiac rhythm. Reduced HRV implies reduced vagal activity. HRV thus has been a balance of parasympathetic and sympathetic activity. HRV was measured by two different methods: time domain and the frequency domain.

Time domain method

Before analysing HRV, non-sinus-originated beats from continuous ECG recording have to be removed. The intervals of two adjacent QRS complexes from sinus node depolarization is called NN (normal-normal) interval.[143] Other variables can be calculated are as follows[143]:

- Mean NN interval
- Standard deviation of the NN interval (SDNN)
- Standard deviation of average NN interval (SDANN)
- Square root of the mean squared differences of successive NN interval (SMSSD)
- The number if NN intervals differences of successive NN interval greater than 50ms (NN50)
- Proportion derived by dividing NN50 by total number of NN interval (pNN50)

Frequency domain method

The frequency domain was calculated by power spectrum analysis which provides the basic information of how power (i.e. variance) distributes as a function of frequency.[143] Three main spectral components were distinguished in a spectrum calculated from short-term recordings of 2 to 5 min: very low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0.04-0.15 Hz), and high frequency (HF, 0.15-0.4 Hz) components. LF is an index of both parasympathetic and sympathetic activities whereas VLF and HF are sympathetic and parasympathetic activities respectively.[190] LF:HF ratio can therefore be an indicator of sympatho-vagal balance. In normal individuals, circadian changes in HRV are present with high HF at night and high LF during daytime.[191]

3.8. Body Composition

3.8.1. Anthropometry

At the onset of the study, height was measured to the nearest 5mm by using a wall mounted stadiometer (Seca 242, Hamburg, Germany). In all measurements, weight was measured as an integral part of Air displacement plethysmography, BodPod procedure (detail on 3.8.2), the methodological validity of which had been established during previous study against calibrated electronic scales.[192] Body mass index was calculated (see Chapter 1). Waist circumference, hip circumference and waist hip ratio were measured according to Table 3.6.[193]

Table 3. 6.   Measurement of the waist, hip and waist-to-hip ratio

1. *Locate the waist.* The waist was located at the smallest circumference of the torso and was not necessarily at the umbilicus. In whom the waist was not present, the smallest horizontal circumference between the 12th rib and the iliac crest was measured.

2. *Measure the waist.* The patient was unclothed at the waist and standing with abdomen relaxed, arms at sides, and feet together. Non-stretchable tape measure was used and care was taken not compress the skin.

3. *Locate the hip.* The hip was located as the maximal posterior extension of the buttocks. If the anterior abdominal wall was sagged, it was included in the measurement.

4. *Measure the hip.* Hip was measured with patient wearing underwear and stood tall but relaxed with arms at sides. Again, non-stretchable tape measure was used and care was taken not compress the skin

5. *Calculate the waist-to-hip ratio.* Divide the waist circumference by the hip circumference

3.8.2. Measurement of Body Volume: Air displacement plethysmography (ADP)

Measurement of body volume is an important contribution to multi-component models of body composition. (See page 74, 3.8.5) Traditionally, body volume has been assessed using hydro-densitometry (under water weighing) using Archimedes's principle.[194, 195] However, this technique requires cumbersome in-house equipment, restricting its availability, and is unsuitable for most patients. Air displacement plethysmography (Bodpod®, Life Measurement Instruments, Califonia, USA), has been developed an alternative technique. It uses the same principle of hydrostatic weighing but uses air instead of water. It measures the volume of air a person's body displaces while sitting inside a chamber. Comparing Underwater weighing and Bodpod, measurement of body density and percent-fat-estimate in different age, sex and BMI groups, showed a strong correlation (r = 0.94 for both, standard error of the estimate = 0.0073 kg/L and 3.58%

respectively, p < 0.001 for both).[196] BodPod had a better precision (standard deviation of differences between duplicate measurements divided by √2) than hydro-densitometry (0.38 kg Vs 0.68 kg respectively for fat mass)[192] and is also suitable to measure a wide range of body sizes.[197]

3.8.2.1.   The basic operating principles

- **Densitometry/density**

With ADP technology, body composition is calculated by body densitometry/density.

Equation 4   Density = Mass / Volume

Densitometry is based on modelling the body into two compartments: a fat compartment and a fat-free mass compartment. The fat-free mass (FFM) compartment consists of protein, water, mineral, and glycogen. (Figure 3.11)

Figure 3. 11.   Schematic diagram of body composition compartments
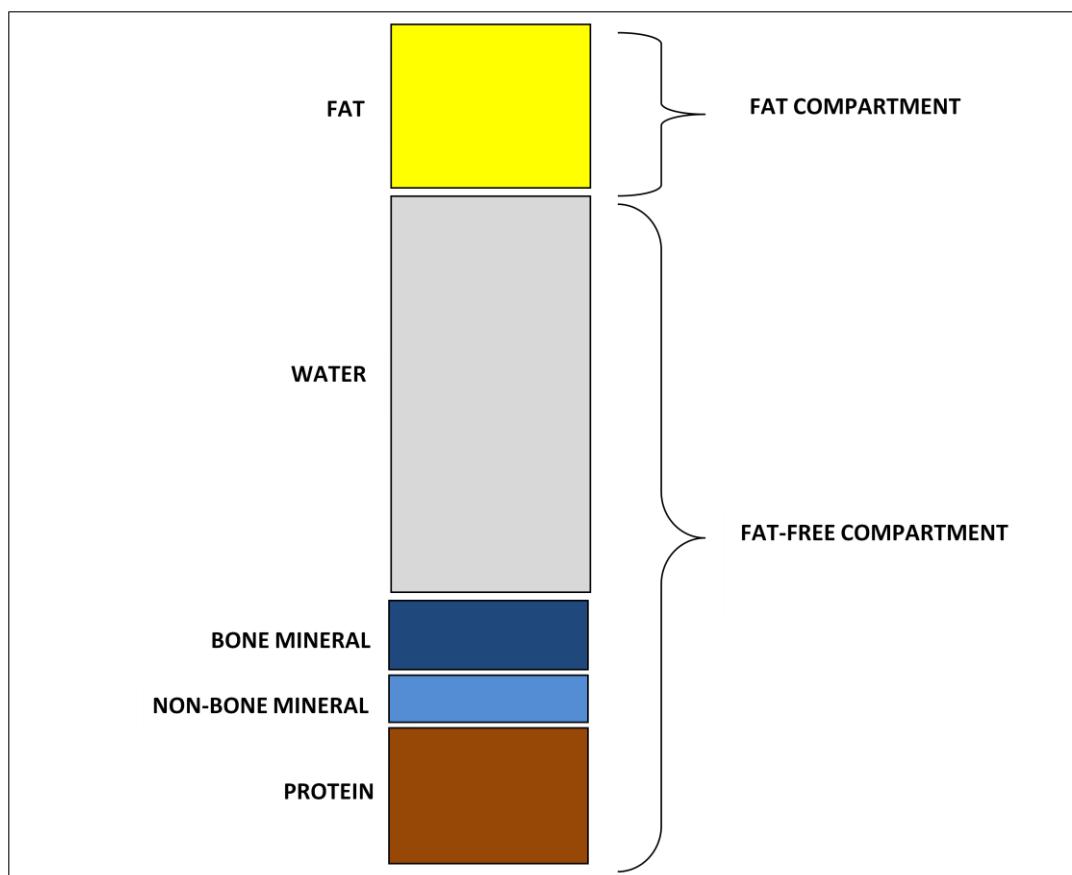
Based on the density of fat and lean tissue, the relative proportion of body fat and lean body mass (fat free mass) was calculated from the subject's body density. A higher density reflects a higher proportion of lean tissue because lean tissue is denser than fat tissue. From whole body density, the percent body fat can be calculated using the equation of Siri[198] as utilised by the Bodpod software.

Equation 5   Percent Fat = (495/ density) – 450

Once the percent body fat is calculated, the percent lean body mass can also be determined as follows:

Equation 6   Percent Lean Body Mass = 100 – Percent fat

- **Mass and Volume Measurement**

Mass was measured by an integrated electronic scale. Body volume was measured by ADP using the principle of Boyle's Law which describes the relationship between the product pressure (P) and volume (V) within a closed system as constant when temperature and moles remain at a fixed measure; both entities remain inversely proportional.[199]

Equation 7 $P_1 V_1 = P_2 V_2$

The ADP measures interior volume (V_2) and pressure (P_2) of the empty chamber directly. Then, when the subject is inside, there is a change in the chamber volume occurred. Hence, body volume (V_1) of the subject can be derived by measuring the pressure (P_1) response to this change.

- **Thoracic gas volume**

The air trapped in the thoracic cavity is not part of the body volume and this must be accounted for as it is included in the raw BV measurement. The manufacturer's software

applies certain corrections to the thoracic gas volume (litres) using equation described by Crapo et al[200]

- **Surface area artefact**

Clothing and hair can contribute significant volume that may result in significant impact on Volume measurements. Therefore, it is important that subjects should wear minimal, form-fitting clothing and a cap to compress the hair on the head.

The presence of the subject in the ADP creates an adiabatic condition because the warmer air, approximately 37°C, is more compressible than the ambient air. Due to this isothermal behaviour, the volume of air in close proximity of the subject's surface can be overestimated. This small surface area artefact (an apparent reduction in the subject's body volume) is automatically calculated by the manufacturer software and provides a subject's correct body volume.

3.8.2.2.   Bodpod® equipment and measurement procedure

All measurements were carried out by using ADP, performed with Bodpod® Body Composition System (Life Measurement Instruments, Califonia, USA) (Figure 3.12 and 3.13). It was a dual-chambered, fibre-glass plethysmograph that determines body volume by measuring changes in pressure within a closed chamber. The front, or test chamber, has a seat that forms a common wall separating it from the rear, or reference chamber. It measures volume of air in the anterior chamber using pressure changes induced by the oscillating diaphragm according to Boyle's laws of relations between volume, pressure, and temperature of gases, and provides raw body volume (raw BV; litres) for each subject simply from the difference between the volumes of air in this chamber, with and without the subject being present.

- Bodpod® was calibrated using a standard phantom of known weight and volume (a cylinder with a nominal volume of 50 l) prior to the measurement of each patient.

- Duplicated measurements were then performed using the Bodpod according to the manufacturer's instructions and recommendations, with each subject wearing tight-fitting swimsuits and swimming caps. A single ADP procedure consisted of two measurements of BV (about 50 seconds each) unless they differed by more than 150 ml, in which case the system required that a third measurement be performed.

- Values for these two raw volume measurements, which were uncorrected for the effects on BV of isothermal conditions created by the subject due to thoracic gas and skin surface area, appeared transiently on the screen during the procedure, and were recorded.

- The thoracic gas volume (litres) was automatically calculated and air volume next to the skin was corrected to achieve the actual body volume. (see above)

- Body weight (mass) and body volume (BV) were used to calculate the body density from which assessment of body composition, particularly fat mass (FM) and fat-free mass (FFM) were calculated. (see 3.8.2.1)

Figure 3. 12.   Principle of Air displacement plethysmography (BodPod)
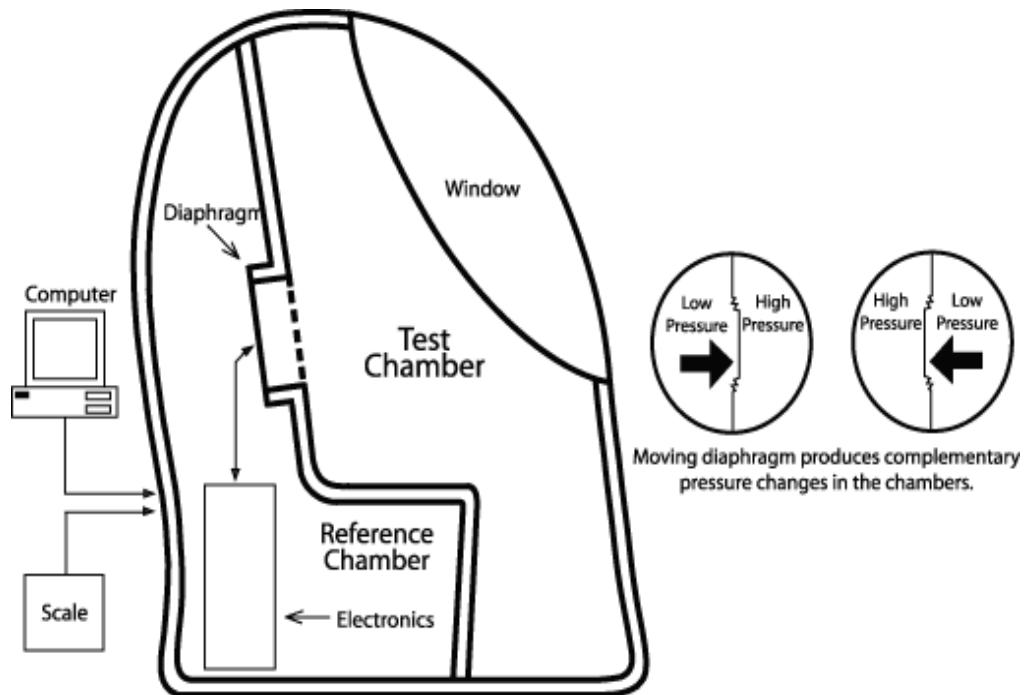
Figure 3. 13.   Body Composition measurement by Bodpod®

3.8.3. Total Body Water

Total body water (TBW) can be measured by an isotope dilution method by administering a known tracer which disperses equally throughout body water and which can be measured.[201] The tracers of choice are stable, nonradioactive isotopes of water containing deuterium or 18-oxygen. Deuterium was used in this study. Patients were fasted overnight and a baseline saliva sample was collected on the morning of measurement for $^2H/^1H$ determination. Then, an oral dose of deuterium oxide (^2H_2O) (0.07 g/kg body weight) was administered and saliva samples were collected at 3, 4, 5 and 6 hour after the dose to capture the period of maximum plateau enrichment of the ^2H_2O within the body. The samples were frozen at -30˙C until they were transferred to MRC Human Nutrition Research (HNR), Cambridge. Measurement of $^2H/^1H$ enrichment in each sample was performed at HNR using isotope ratio mass spectrometry[202] which allowed the precise measurement of stable isotope.[203] ^2H_2O dilution space was determined using the following equation[204]

Equation 8 $^2H_2O \text{ (kg)} = [D \times T \text{ (Ed-Et)} / d \times \text{(Es-Ep)}] / 1000$

D (g)	the amount of oral dosing solution administered to the subject
T (g)	the amount of deionised tap water used to dilute a sample of the dosing solution d (g).
	All expressed as delta value (‰)
Ed	the enrichment of the diluted dose d in T
Et	the enrichment of the tap water diluent
Es	the mean enrichment of the post dose samples
Ep	the mean enrichment of the pre dose sample

Total body water (TBW in kg) was calculated by reducing ^2H_2O dilution space values by 4% to account for the exchange of deuterium with non-aqueous hydrogen[205] and then Lean Body Mass (LBM) calculated as:

Equation 9   LMB (kg) = TBW/ hyd

Where *hyd* represents an assumed hydration fraction of 73% for LBM.[206] Finally, fat mass (FM (kg)) was calculated as the difference between weight and LBM:

Equation 10   FM = Wt - FFM

And % TBW and % Fat was calculated as a proportion of body weight.

3.8.4. Dual Energy X-ray Absorptiometry

3.8.4.1.   Background

Bone mineral density was previously measured by a single photon-emitting radioactive source and a scintillation counter positioning on the other side of the wrist. The number of detected counts was related to the amount of attenuating calcium or bone present. This single photon system evolved to a dual-photon system, now based on a filtered x-ray source and referred to as dual-energy x-ray absorptiometry (DXA). It is a non-invasive technique that measures the differential attenuation of two X-rays as they pass through the body (Figure 3.14Figure 3. 14) and permits measurements of body composition.   It distinguishes bone mineral from soft tissue and subsequently divides the latter to FM and fat free soft tissue[207, 208] based upon the following principles:

- X-ray attenuation increases with increasing atomic number. Relative to lean tissue (water, protein, and carbohydrate), fat contains a higher proportion of carbon

(atomic no. = 6) to oxygen (atomic no. = 8) and thus has lower average atomic number. Therefore, lean tissue has a higher X-ray attenuation than fat, and this differential attenuation is greater for high- than for low-energy X-rays.

- The ratio of high to low-energy X-ray attenuation coefficients (DXA ratio or R value) is the fundamental DXA measurement employed to calculate body composition. Using "R" value, mass fraction of each component in two compartments can be determined. R value for the soft tissue (lean and fat) is directly proportionate to the amount of fat in the tissue.[209]

- Non-skeletal tissue is assigned to fat and lean compartments, and the differential attenuation of low- and high-energy X-rays is employed to calculate the relative proportions of fat and lean tissue.

Figure 3. 14.   Body Composition measurement by DXA scan

The data may also be analysed to yield information about the composition of the whole body or of individual segments (Figure 3.15 and 3.16), e.g., limbs or abdominal regions.[208] The precision errors (1SD) for total body bone mineral density, percent fat,

fat mass and lean tissue mass were 0.01 g/cm^2, 1.4%, 1 kg and 0.8 kg respectively.[208] However, one of the limitation of DXA is the size of the scanning area (approximately 190 x 60cm) and weight limit (130Kg for the machine used in the present study). For this reason, it cannot be used in the very obese patients. The manufacturer, GE Lunar cooperation, Maidson, Wisconsin, has recently developed a newer machine to overcome this issue.

3.8.4.2.   Equipment and measurement procedure

Prodigy Bone densitometry (GE Lunar, Madison, WI) was used to measure whole body FFM, consisting of lean mass and bone mineral content (BMC), and FM.

- Subjects lay on the table while the scanner arm passes over them in a rectilinear manner. The typical scan duration was 10–12 min, depending on the height of the subject while the DXA scanner performed multiple fast speed transverse scans from head to toe with 1 cm intervals. The radiation exposure per scan was estimated to be 1 μSv, which was lower than the average daily background radiation level in UK (7 μSv). The software package was used by only one member of the investigator team.

- BMC, FM and lean mass were derived according to computer algorithms provided by the manufacturer (Lunar software version 8.1). Body weight was obtained by adding lean mass, BMC and FM. Percentage body fat was calculated as FM relative to body weight. FFM was computed as the sum of lean mass and BMC. Bone mineral density (BMD) is BMC normalised for bone size and is expressed as grams per centimetre squared.

- Subjects who did not have DXA measurement at baseline (exceeded weight limit of >130 kg) had DXA in their 6 week visit if they achieved a body weight below 130kg. The BMC value of week 6 measurement was used for four compartment model assuming no change during acute weight loss period of 6 weeks.

- Subjects, in whom their body size (the width) exceeded the scanner area, were scanned without part of left upper limb. The data was analysed for both measured total and individual segments of FM. FM measured on the area of part of that limb was subtracted from and whole FM. Then, FM of the right upper limb area was

added to FM to achieve total body FM assuming FM is equal between left to right upper limb. Example of such a calculation is shown in Figure 3.17.

Figure 3. 15.   Dural Energy X-ray Absorptiometry (DXA)

Left panel illustrates the bone mineral component whereas right panel showed the soft tissue component. The various lines across indicate different regions and separate body composition of these regions were available for analysis.
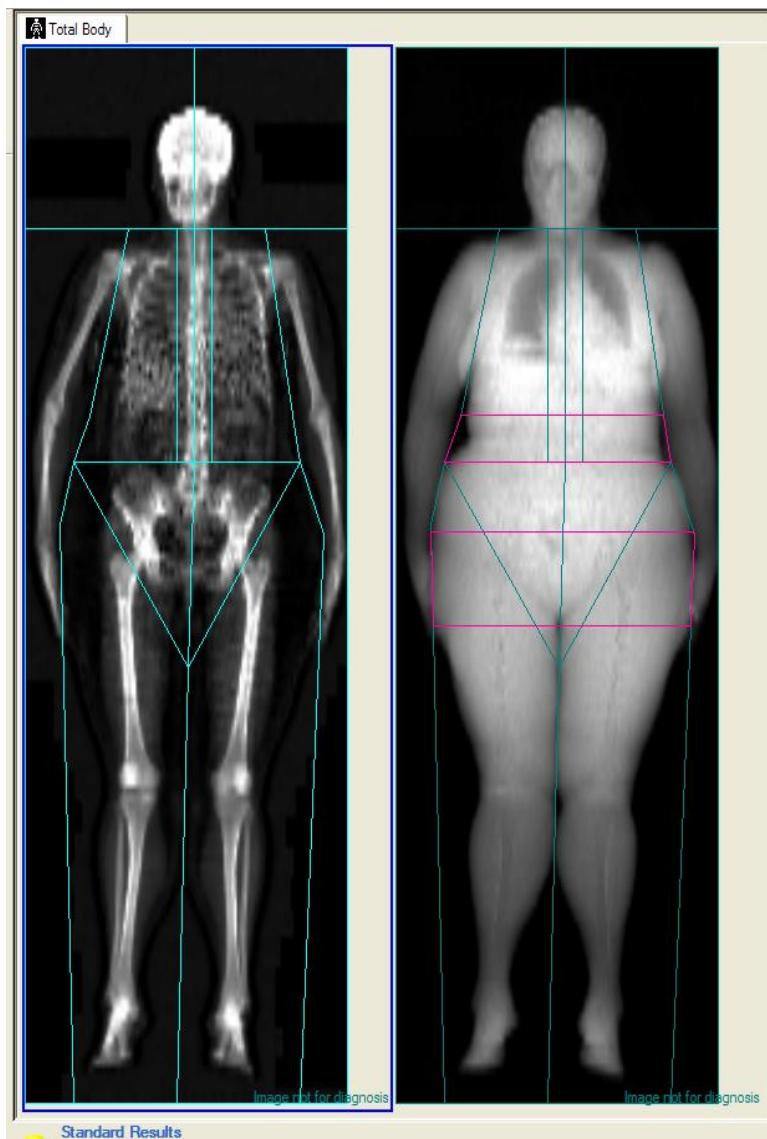
Figure 3. 16. Body composition data of the whole body and individual segments derived from DXA
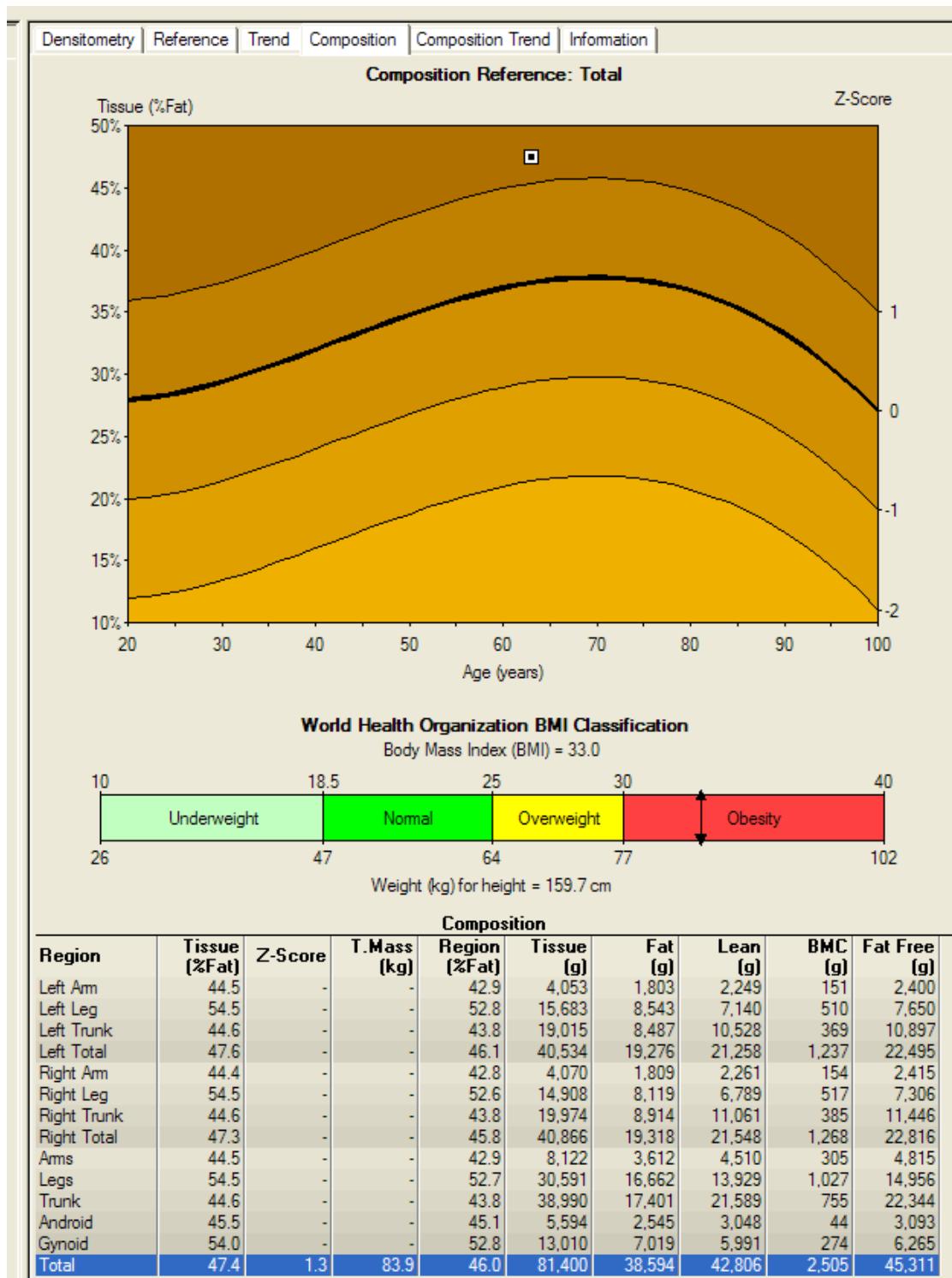
Figure 3. 17. Calculation of a body composition in a patient in whom the body width exceeds the scanning area.

Region	Tissue (%Fat)	Z-Score	T.Mass (kg)	Region (%Fat)	Tissue (g)	Fat (g)	Lean (g)	BMC (g)	Fat Free (g)
Left Arm	34.4	-	-	33.6	2,030	699	1,331	48	1,379
Left Leg	35.9	-	-	34.4	17,354	6,226	11,128	738	11,866
Left Trunk	43.7	-	-	42.9	29,953	13,097	16,856	552	17,408
Left Total	39.5	-	-	38.3	51,999	20,522	31,477	1,597	33,074
Right Arm	33.7	-	-	32.4	5,596	1,886	3,710	222	3,932
Right Leg	35.9	-	-	34.5	17,676	6,345	11,331	739	12,069
Right Trunk	43.7	-	-	42.8	26,608	11,626	14,981	547	15,528
Right Total	38.9	-	-	37.6	52,216	20,292	31,924	1,732	33,656
Arms	33.9	-	-	32.7	7,626	2,585	5,041	270	5,311
Legs	35.9	-	-	34.4	35,030	12,571	22,459	1,477	23,936
Trunk	43.7	-	-	42.9	56,561	24,724	31,837	1,098	32,936
Android	50.3	-	-	50.0	9,385	4,721	4,664	53	4,717
Gynoid	40.4	-	-	39.4	17,225	6,958	10,267	429	10,696
Total	39.2	3.6	107.5	38.0	104,215	40,814	63,401	3,329	66,730

It was assumed that the composition of left and the right arm area were the same. Fat mass (FM) from the area of left arm (➡ 699g) was subtracted from the measured total fat mass   (➡40814g). Then FM from right arm area (➡ 1886g) was added to the measured total FM to achieve patient actual total fat mass (42001g). Similar calculation was made for the others: lean, BMC and fat free mass.

3.8.5. Body composition Models

Basic two-compartment model measures fat and fat-free mass. Its use was limited by assumptions regarding the consistency of composition of fat free mass. The FM, which is defined as chemically extractable fat, is assumed to have a density of 0.9007 g/cm^3 and be anhydrous,[210] whereas the FFM is regarded as having a density of 1.1000 g/cm^3 and a water content of 73.72%.[211] To overcome this limitation, Siri[198] proposed a three-compartment model. This model measures the three major distinct chemical components within body: fat, water and the remaining fat-free dry mass (FFDM). It is based on the measurements of both body density and total body water while a constant mineral-to-protein ratio of 0.35 is assumed. It overcomes some uncertainties concerning the hydration fraction of fat free mass.[211] Taking into account the various assumed densities of the 3 components and the assumed constant ratio of protein to mineral, FM was calculated from the basic measurements as follows:

Equation 11   FM (kg) = [(2.220 x BV) – (0.764 x TBW)] – (1.465 x BW)

BV is body volume in litres, TBW is in litres, and BW is body weight in kilograms.

The four-compartment model was based on principles similar to those adopted for the three-compartment model, but involved additional measurement of bone mineral by DXA, allowing the body to be segregated into fat, water, mineral and protein.[212] The density of fat-free mass (D_{ffm}) was calculated from the mass and volume of each individual component (water, protein and mineral). Fuller et al[212] showed that there is a considerable inter-individual variation in the density of fat-free mass. This model, therefore, is more robust in minimising inter-individual variability in the composition of FFM.

Equation 12   FM (kg) = [(2.747x BV) – (0.710 x TBW)] + [(1.460 x A) – (2.050 x BW)]

where A is BMC determined by DXA (in kg). Total-body mineral mass was calculated as BMC x 1.2741.

This model was used for the body composition measurement in the study as it is now well established and widely used method of measuring body composition.[213]

Figure 3. 18. **Schematic diagram showing body composition models**

(FFM =fat free mass, FFDM= fat free dry mass, A= 2 compartment model, B= 3 compartment model, C= 4 compartment model)
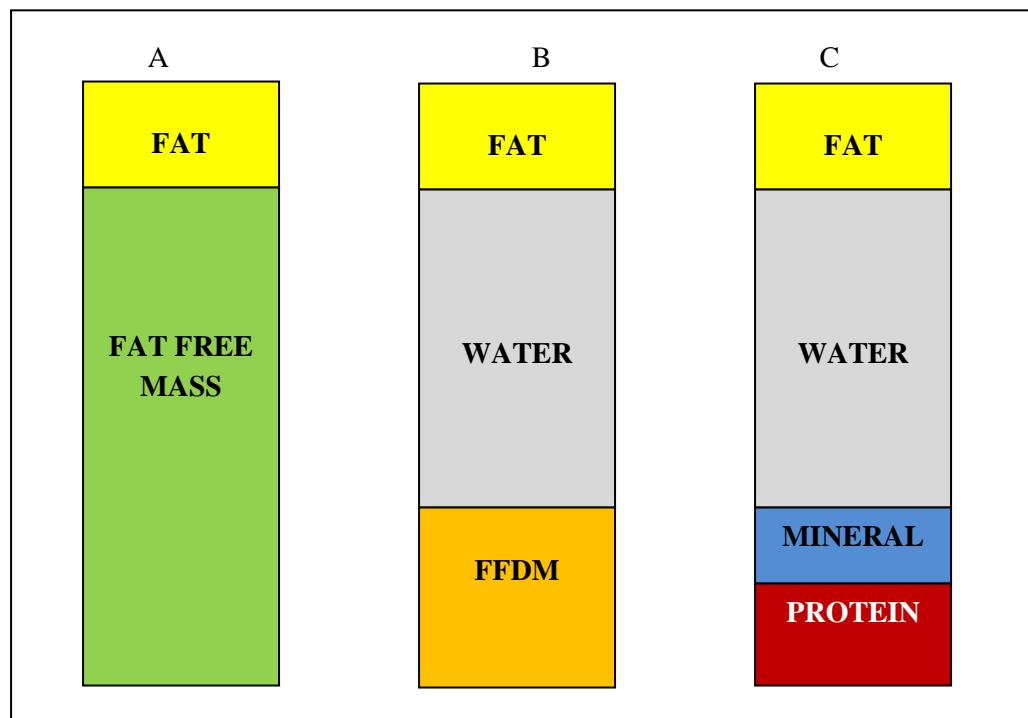
3.8.6. Body composition with whole body quantitative MRI

This is a novel quantitative magnetic resonance (QMR) methodology (EchoMRI-AH, Echo Medical Systems, LLC, Houston, TX USA) for measurement of whole-body fat and lean mass in humans. It uses the differences in the nuclear magnetic resonance (NMR) properties of hydrogen atoms in organic and non-organic environments to fractionate signals originating from fat, lean tissue and free water.[214] QMR measures body composition in live, un-anaesthetised small animals, and has become the method of preference for measuring their fat mass. It has been reported that changes in fat and

lean mass in mice weighing typically 25g can be measured with a coefficient of variation that ranges from 0.34% to 0.71%.[214] This technology has now been scaled for adult human application (EchoMRI-AHTM). This device is designed to measure total body fat, lean mass, body fluid, and total body water in adult with body weight from 60 to 250kg.[214] The device is based on magnetic resonance principle i.e., no ionizing radiation.

3.9.   Biochemistry

Venous blood was drawn in the fasting state and plasma was immediately extracted and stored at -20 C.

3.9.1. B-type Natriuretic Peptide (BNP)

Plasma BNP concentration was measured at rest and at the recovery phase of exercise testing. It was repeated at week 6 and 16. Triage® BNP test kid (Biosite® Incorporated, California) was used for analysis. It is a single use fluorescence immunoassay device design to determine the concentration of BNP in EDTA-anticoagulated plasma samples. All the tests were performed in one batch at the end of the study.

3.9.2. Plasma norepinephrine level

Resting plasma norepinephrine levels were measured by the High Performance Liquid Chromatography electrochemical (HPLC- EC) technique (Figure 3.19). This is a well-established assay used for daily clinical practice. This method of analysis relies on extraction of catecholamines from plasma by their absorption to solid alumina at pH 8.6. Subsequent elution at pH 4 and injection onto an HPLC system allows separation of analytes of interest by their varying retention on a C18 column, the time separated catecholamines are then detected by oxidation in the cell of the electrochemical detector, the resulting current produced being proportional to the concentration of analyte. Standard solutions containing known concentrations of catecholamines are used to calibrate the detector, allowing the calculation of levels in patient and QC samples. A

non-naturally occurring catecholamine, dihydroxybenzylamine (DHBA) is used as an internal standard to correct for any losses in the extraction procedure.

Figure 3. 19.   High Performance Liquid Chromatography Standard
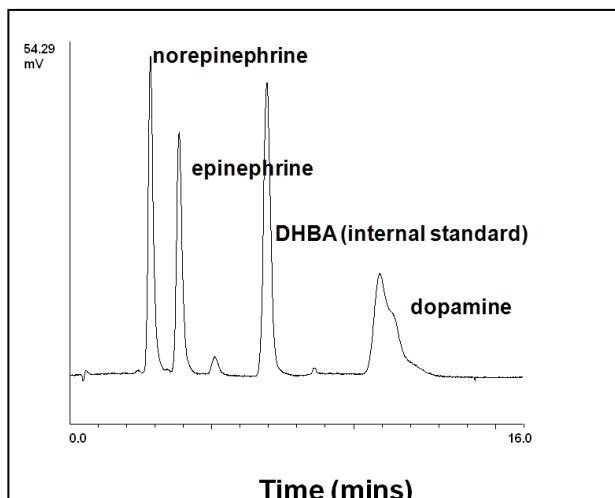
3.9.3.   Highly sensitive C-reactive protein

C reactive protein was an acute phase protein secreted by hepatocytes in response to infection and inflammation.[215] CRP was a moderate predictor of coronary heart disease (CHD). CRP > 2mg/l was predicted to be associated with higher CHD comparing with a group with CRP of <0.78 mg/l (odd ratio 1.92, with 95% CI of 1.68 to 2.18).[216]

Standard CRP assay measures the CRP levels which are increased up to 1000 folds. However, the assay was not sensitive enough to detect normal range and therefore not suitable to use as a predictor of cardiovascular risks.[217] Subsequently, newer highly sensitive CRP (hsCRP) assays have been developed with a lower limit detection level of 0.1 mg per litre.[218, 219] In the present study, CRP were measured by using high-sensitivity, two-site enzyme linked immunoassay (ELISA). This assay was similar to the one used in west of Scotland coronary prevention study .[220] It uses a peroxidase-conjugated rabbit antihuman C-reactive protein antibody (DK2600, Dako, Glostrup,

Denmark) and a polyclonal anti–C-reactive protein capture antibody. The lower limit of the working range of the assay was 0.1 mg per litre.

3.9.4. Antioxidant Profile

Oxidant stress plays a critical role in the pathogenesis of endothelial dysfunction.[221] Systemic processes that invoke endothelial dysfunction include a stress-induced activation of intracellular oxidative signalling, with secondary modulation of LDL oxidation, nitric oxide (NO) bioavailability and vascular inflammatory gene expression.[222] These processes may be modulated by factors such as obesity, weight loss and dietary composition (macronutrient and micronutrient intake).[223] Obesity has been associated with enhanced oxidant stress.[222, 224] The possible causes include increased oxygen consumption, increased fat deposition, diminished antioxidant capacity and an increased rate of free radical formation i.e. superoxide anion.[225] These abnormalities have been shown to be reversible with weight loss.[225, 226] Oxidative stress can be measured by different methods including lipid peroxidation, total antioxidant status (TAS), erythrocyte-reduced glutathione, erythrocyte superoxides dismutase and LDL oxidation. In this study, ferric reducing ability of plasma (FRAP) assay was used to assess TAS i.e., the total reactive ('antioxidant') reactive capacity of the biological fluid. The FRAP assay was developed by Benzie and Strain in 1996.[227] It was based on the principle that in low pH of 3.6, reduction from ferric (Fe^{+++}) to ferrous (Fe^{++}) ion causes formation of coloured ferrous-tridyltriazine complex. The absorbance increase at 593nm in test reaction mixture were compared with those of known ferrous ion concentration to provide FRAP value.[227] The higher the FRAP value, the higher the TAS.

3.9.5. Other Biochemistry

Urea and electrolytes, liver function test, fasting lipid profile, fasting glucose, fasting insulin level for HOMA model,[228] HbA1C, adiponectin, leptin and TNFα were measured at baseline and each measurement visit.

Fasting glucose, Insulin and HOMA model

Insulin was assayed in singleton on a 1235 Auto DELFIA (Perkin Elmer Life Sciences (Wallac Oy, Turku, Finland) automatic immunoassay system using a two-step time resolved fluorometric assay (Kit No. B080-101). All reagents, standards and consumables are those recommended and supplied by the manufacturer. A series of lyophilised calibrators are supplied with the kit. The calibrators are referenced to WHO 1st IRP 66/304. The Lower limit of detection was 1.3 pmol/l. Between batch imprecision were 3.1% at 29 pmol/l , 2.1% at 79.4 pmol/l, 1.9% at 277 pmol/l & 2.0% at 705 pmol/l.[229] HOMA model (HOMA2) was downloaded from http://www.dtu.ox.ac.uk/homacalculator/index.php; Diabetes Trial Unit, the Oxford Centre for Diabetes, Endocrinology and Metabolism. Fasting glucose and insulin values were required for to calculate the HOMA2.

Adiponectin and Leptin

Adiponectin is an adipokine secreted from matured adipocytes.[230] Adiponectin acts as a insulin sensitiser and exerts anti-inflammatory activites.[231] Low adiponectin levels were reported in diseases with accelerated atherosclerosis (insulin resistant, type 2 diabetes, obesity, coronary artery disease)[232-234] and an important predictor of cardiovascular event.[235]

Leptin is an adipokine with a 167-aminoacid peptide. Circulating leptin levels increased with obesity and correlates strongly with the percentage body fat.[236] Studies reported correlations of leptin levels with established CVD (cholesterol, blood pressure),[237, 238] vascular dysfunction,[239] and inflammation.[240]

In the present study, adiponectin and leptin were assayed by two-site microtitre plate-based DELFIA[241] with a lower limit of detection at 30 ng/ml for adiponectin and 0.1ng/l for leptin. Antibodies and standards reagents were supplied by R&D Systems

(R&D Systems Europe, Abingdon, UK). Between-batch imprecision for adiponectin were 5.4% at 3.6 µg/ml, 5.2% at 9.2 µg/ml and 5.8% at 15.5 µg/ml. The assay measured 'total' adiponectin. The relative reactivity of the various forms of adiponectin found in human serum was not known with this assay. Between-batch imprecision for leptin were 7.1% at 2.7 ng/ml, 3.9% at 14.9 ng/ml and 5.7% at 54.9 ng/ml.

Interleutin-6

Interleukin 6 (IL-6) is a cytokine produced in various tissues in response to tissue injury or infection. IL-6 exerts proinflammatory effects and stimulates the liver to produce positive acute-phase proteins. High levels of IL-6 have been shown to be associated with high CHD risk.[242]

In the present study, IL-6 was assayed by a two-site microtitre plate-based enzyme linked immuno-solvent assay (ELISA) with the reagent supplied by R&D Systems (R&D Systems Europe, Abingdon UK). The lower limit of detection of the assay was 0.7 pg/ml. Imprecision Data between-batch were 3.4% at 29 pg/ml, 8.8% at 157 pg/ml and 8.2% at 203 pg/ml.

Tumour necrosis factor- alpha

Tumour necrosis factor- alpha (TNF-α), is a pleiotropic adiopokine. The increased level of TNF-α promotes a proinflammatory state, impair the preadipocytes differentiation and induce insulin resistance[243] and endothelial dysfunction.[244] In the present study, TNF-α was assayed by quantitative ELISA technique with the reagent supplied by R&D Systems (R&D Systems Europe, Abingdon UK). The mean lower limit of detection of the assay was 0.391 pg/ml. Imprecision Data between-batch were 7% at 5.97pg/ml, 8.3% at 37.4pg/ml, 6.4% at 282pg/ml and 6.3% at 3869 pg/ml.

3.10. Statistical analysis

Data were analysed using SPSS software (version 12) and GraphPad Prism software. Changes of peak VO2 (primary end point) and other various parameters at week 0, 6 and 16 were analysed by repeated measure ANOVA.

This particular statistical test was used with the assumption that

1. matching of variables would be effective
2. subjects would be independent
3. random variability would be distributed according to a Gaussian distribution
4. comparisons would be made over 3 points in time that were defined by one factor (weight loss intervention)

The primary end point was powered and analysed on the assumption that data would be normally distributed because

1. No a priori reason to expect a skewed or bivariate distribution because entry criteria were tight.
2. The Kanoupakis et al182 paper that was used for the sample size calculation of our study was reported used parametric tests.
3. The planned sample size (N=24) would also make it likely that parametric tests be more appropriate.

Further exploration was made by *post hoc* contrast testing with Bonferroni's adjustment. Bivariate correlation analysis was calculated to assess a possible relationship between peak VO_2 and other study variables of prognostic indicators of heart failure; and between fat mass and other variables including haemodynamic parameters and biochemistry. Relationships between variables were assessed by Pearson's linear correlation. Bland- Altman analyses were used to compare two different techniques of measurement of fat mass. Fat mass difference Vs mean fat mass between 4C and QMRI. Fat mass and fat free mass changes over time was analysed by using 2 way repeated measure ANOVA. Data were presented as mean±SD. Range was given when relevant.

CHAPTER 4:

RESULTS

CHAPTER 4:   RESULTS

The result will be described as following:

- Baseline characteristics

- Body composition changes

- Cardiac performance, haemodynamic changes and sympathetic activity

- Metabolic profile and biochemical parameters

4.1.   Baseline characteristics - Results

A total of 14 patients were recruited, of whom 11 patients completed the study. Three patients withdrew from the study (two could not follow strict diets and one was diagnosed with breast carcinoma.) The mean age was 54.7 ± 9.7 years and mean BMI was 38.28 ± 4.73kg/m^2 (range 33.31-48.21). Three patients (33%) were morbidly obese (BMI >40 kg/m^2) and all of them were also diagnosed with hypertension. The background cardiovascular risk profile and treatments are shown in Table 4.1. As recruitment was mainly carried out in diabetes clinic, 8 patients (73%) had underlying type 2 diabetes. Among them, one patient was diet controlled, one was on insulin alone, 4 were on oral anti-diabetes drugs and two on both oral glucose lowering therapy and insulin. The daily insulin requirements were 37, 86 and 95 units/day at baseline. The first patient managed without insulin by week 16. The insulin requirement for the latter reduced to 8 and 32 units by week 16. Eight patients (88%) met the international diabetes Federation (IDF) criteria for metabolic syndrome. Baseline demographics, body composition, haemodynamic characteristics and baseline biochemistry of the 11patients who completed the study, were demonstrated in Table 4.2 and 4.3.

Table 4. 1. **Background cardiovascular risk factors and pharmacotherapy (N=11)**

	Number of patients (n) Total (male)
Cardiovascular risk factors	
Heart failure	4 (2)
Hypertension	8 (6)
Ischaemic heart disease	3 (2)
Type 2 diabetes	8 (6)
Dyslipidaemia	8 (7)
Pharmacotherapy	
Heart failure medication	3 (2)
Antihypertensive therapy	5 (1)
Insulin therapy	3 (1)
Oral anti-diabetic therapy	6 (5)
Statin therapy	4 (4)

Table 4. 2. **Baseline demographics, body composition and haemodynamic characteristics**

	N=11
Age (years)	54.7±9.7 (42-68)
Body weight (kg)	112.7±23.25 (84.2-148.3)
BMI (kg/m^2)	38.28±4.73 (33.31-48.21)
Waist circumference (cm)	121.7±13.75 (99-145)
Fat mass (kg)	50.58±14.86 (33.17-80.03)
Fat free mass (kg)	62.29±12.58 (43.35-83.97)
Systolic BP (mmHg)	142.7±19.26 (119-173)
Diastolic BP (mmHg)	81.55±9.92 (63-100)
Resting heart rate (bpm)	76.18±16.94 (56-109)
Left ventricular ejection fraction (%)	49±19.15 (29-64)
Left ventricular mass (g)	296.5±74.67 (172-431.5)
Peak VO$_2$ (ml/kg/min)	18.33±4.53 (12.3-27.5)

Values represent means ± standard deviation, ( ) indicates range.

Table 4. 3.   Baseline Biochemistry

	N=11
Fasting glucose (mmol/l)	7.436±2.66 (4.1-13.3)
HbA1c (%)	6.88±1.3 (5.5-9.3)
Fasting insulin (pmol/l)	83.63±44.31 (48-188)[*]
Cholesterol (mmol/l)	4.2±0.60 (3.50-5.50)
Triglyceride (mmol/l)	1.9±0.88 (1.0-3.8)
HDL Cholesterol (mmol/l)	1.05±0.27 (0.68-1.49)
LDL cholesterol (mmol/l)	2.34±0.58 (1.62-3.33)
ALT (U/L)	41±20.81 (16-88)
C-reactive protein (ng/l)	4.45±3.30 (0.66-9.7)
TNF-α (pmol/l)	3.87±1.68 (1.90-6.78)
Adiponectin (µg/ml)	4.63±2.34 (1.5-7.3)
Leptin (µg/l)	52.87±48.88 (12.9-174)
24h urinary NE (nmol/24h)	396.8±254.5 (132-975)
Plasma norepinephrine (ng/l)	435±142.4 (227-686)
Plasma BNP (pg/ml)	74.09±109.8 (5-303)

Values represent means ± standard deviation, ( ) indicates range. * was based n=8 (excluded patients treated with insulin)

4.2.   Baseline characteristics – Discussions

Our cohort had high proportion of patients with diabetes (88%). Among them, 50% was recruited from the diabetes clinic and the rest from elsewhere. This reflected that a high prevalence of diabetes in patients with high cardiovascular risk group. Unfortunately, only 4 patients (33%) were diagnosed heart failure prior to the recruitment. Therefore, the mean LVEF was not very low at 49%. Only 3 patients had LVEF <45% at baseline. None of our patients had a level of NE above the poor prognosis cut off (>600pmol/l). This reflected our recruitment exclusion criteria of severe heart failure group (NYHA grade IV). Two out of four patients with heart failure had pVO_2 lower than the cut off criteria (14 ml/kg/min) for heart transplantation at baseline (13.1 and 12.3 ml/kg/min). In fact, the later patient was previously on cardiac transplant list and then taken out from the list as her clinical symptoms had improved with anti-heart failure therapy. With the advanced in medical therapy, patients were remarkably asymptomatic despite their advanced disease. Similarly, one patient was excluded from the study after screening due to very poor LVEF of <15% and had very low peak VO_2 at 10ml/kg/min. Surprisingly he managed to perform cardiopulmonary exercise testing to Naughton stage 3 and his NYHA was stage II.

4.3.   Weight loss intervention – result

LELD with a calorie of 800-100kal was well tolerated. 12 out of 13 patients completed Liquid phase of intensive dietary programme (initial 6 weeks). Only one patient could not tolerate the diet and dropped out from the study. One patient, who completed the liquid phase, withdrew from the study at week 7 following the diagnosis of breast cancer. One patient chose the commercial nutrition supplement (Slim Fast®, Surrey, UK) than milk. All patients lost weight with a range from 6.6 kg to 23 kg at 6 weeks and 4.9 to 32.1 kg at week 16 (p <0.0001) (Figure 4.1). Detailed body composition changes were demonstrated in section 4.5.

Figure 4. 1.   Weight loss in individuals at week 6 and week 16
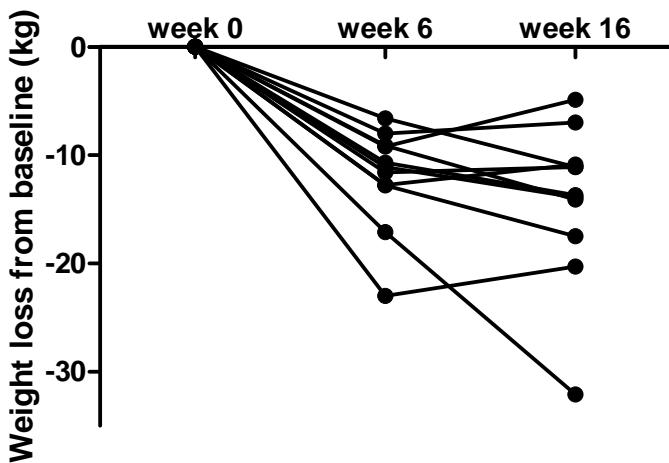
4.4.   Weight loss intervention – Discussion

This study suggested that the use of LELD with calories of 800- 1000kcal is safe in inducing acute weight loss in obese patients with heart failure and or high cardiovascular risk, particularly in the group of type 2 diabetes treated with insulin. Patients generally tolerated the diet well apart from minor side effects, i.e., constipation and diuresis. Indeed exercise tolerance also improved with weight loss. However, regular close follow up was needed to manage the haemodynamic and metabolic changes that required adjustment of therapy for their underlying co-morbidities. Weight loss in patients with diabetes were generally more difficult than patients without diabetes.[245] One possible explanation would be the potential risk of hypoglycaemia with glucose lowering therapy following a restriction of calorie/carbohydrate intake. In addition, most of the glucose lowering agents including insulin, sulphonyurea and thiozolidinediones were known to be associated with weight gain.   In the present study, glucose monitoring was closely supervised and glucose lowering therapy was actively titrated down. It proved that patients with diabetes could loose weight effectively if they were able to restrict to their energy intake providing energy deficit. Orlistat used in the later phase of the study aided to maintain the weight. All patients had tolerated orlistat well without gastrointestinal side effect. This was achieved by patients following a strict reduction of dietary fat intake below 70g per day.

4.5.   Body composition changes - Results

All results were expressed as changes at week 6 (acute weight loss) and week 16 (weight loss maintenance) comparing to the baseline. As stated above, following the intensive diet, a significant mean±SD weight loss of 12 kg±4.6 was achieved at 6 weeks and maintained until the end of study (mean 14.2 kg±7.3, p <0.0001) (Figure 4.2). Similarly, there was a significant reduction in mean waist circumference from baseline (10.55 cm±5.55 and 14.05 cm±7.38 at week 6 and week 16 respectively, p<0.0001) (Figure 4.2) but there were no changes in waist/hip ratio. The detail changes in body composition analysis at week 6 and week 16 are demonstrated in Table 4.4. With weight loss, there were significant reductions in all components of body composition: FM (8.04 kg±3.23 and 11.21 kg±5.82), FFM (4.17 kg±2.05 and 3.24 kg±3), total body water (TBW) (3.88 kg±2.55 and 3.34 kg±3.47) and fat free dry mass (FFDM) (1.02 kg±0.43 and 1.05 kg±0.96) at week 6 and 16 respectively (p<0.0001 for first three parameters and <0.0003 for the last one). Therefore, FM loss was much greater than the FFM loss (22.14% *Versus* 5.23% at week 16). There was an initial mean 4.17 kg loss of FFM at the end of weight loss phase (week 6), 0.93 kg of which was then regained at week 16 (Figure 4.3). When the percentage body composition changes were measured against total body mass, reductions in the percent FM were seen whereas significant increases in other compartments; percent FFM, TBW and FFDM at week 6 and week 16 resulting in more favourable changes i.e., a healthier body composition.

Figure 4. 2.   Changes in body mass and waist circumferences
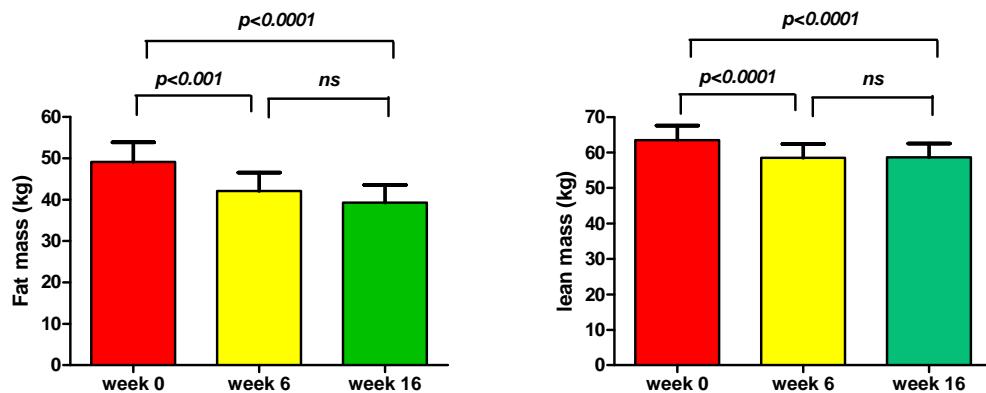
Bar graph representing means and standard error of means. N=11. The omnibus significance for the trend was *p<0.0001*. The significant was determined by 1-way ANOVA repeated measures, and *post hoc* testing with Bonferroni's adjustment.

Table 4. 4.   Effect on body composition following weight loss and weight loss maintenance

Body composition	Baseline	Week 6	Week 16	P value
Total mass, kg	112.7±23.3	100.7±20.2*	98.5±22.2*	<0.0001
FM, kg	50.6±14.9	42.5±13.6*	39.4±15.1*	<0.0001
FFM, kg	62.29±12.6	58.12±11.1*	59.05±11.7*	<0.0001
TBW, kg	47.9±9.9	44±8*	44.6±8.7*	<0.0001
FFDM, kg	14.62±2.7	13.6±2.8*	13.57±2.9*	<0.0003
Percent FM (%)	44.35±5.9	41.95±6.9	39.47±8.5*	<0.0003
Percent FFM (%)	55.49±7	58.65±8.6	61.68±1.8*	0.0003
Percent TBW (%)	42.4±4.1	44±4.6	45.7±5.8*	0.0009
Percent FFDM (%)	13.05±1.5	13.66±1.7	14.06±1.7*	0.0032

Values represent mean ± standard deviation. N=11. Significance was determined by one way ANOVA with repeated measure. When the omnibus test was significant (final column), Bonferroni multiple comparisons was performed for baseline *versus* week 6, baseline versus week 16. Significant difference is indicated by * ($p < 0.01$). There was no significant difference between week 6 and week 16 for any of the parameters.

FM= fat mass, FFM = fat free mass, TBW = total body water, FFDM = fat free dry mass.

Figure 4. 3.   Comparison of fat mass Vs fat free mass loss/gain (4C model)
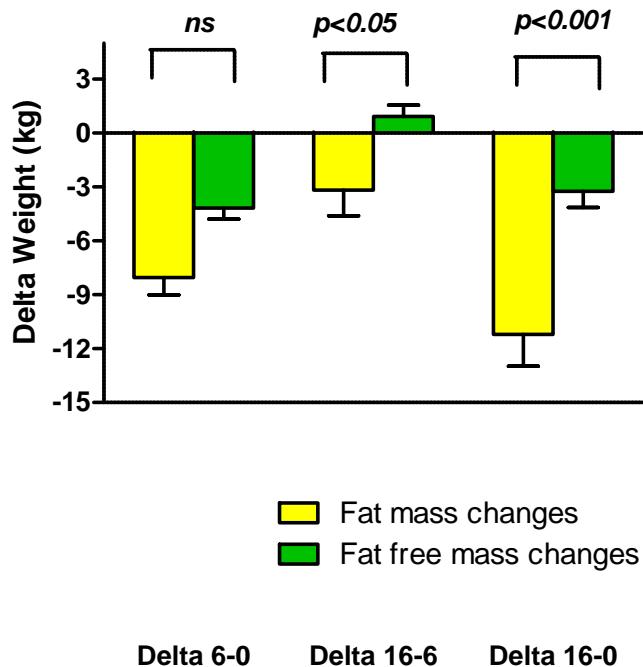
- Delta 6-0 = mass changes between week 6 (at the end of acute weight loss phase) and week 0 (baseline),
- Delta 16-6 = mass changes between week 16 and 6
- Delta 16-0 = mass changes between week 16 and 0

Bar graph represents means and standard error of means. N=11. The omnibus significance for interaction between fat mass and fat free mass changes was *p=0.015* and *post hoc* testing with Bonferroni's adjustment.

The body composition measured by a newer technique i.e., quantitative MRI (QMR) was shown in Figure 4.4. This method also demonstrated a significant loss in both FM and FFM (7.05 and 9.86 kg of FM, and 4.95 and 4.84 kg of FFM at week 6 and week 16 respectively; p<0.0001 for all).

Figure 4. 4.   Fat mass and fat free mass changes measured by quantitative MR
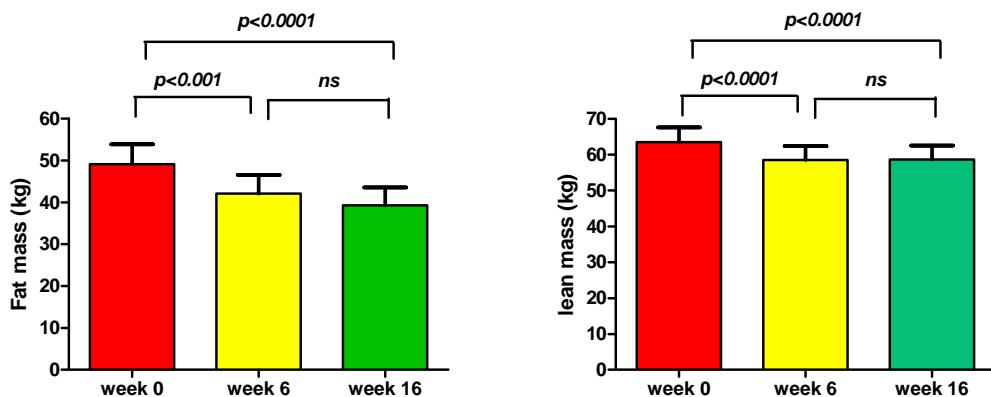
Bar graph representing means and standard error of means. N=11. The omnibus significance for the trend was *p<0.0001*. The significant was determined by 1-way ANOVA repeated measures, and *post hoc* testing with Bonferroni's adjustment.

The relationship between QMR estimates of absolute fat mass and those obtained with the 4C model was illustrated in a correlation plot (Figure 4.5).The Pearson correlations were high at 0.98, 0.99 and 0.98 for week 0, week 6 and week 16 data respectively. However, high correlations were not sufficient to demonstrate agreement because they were strongly influenced by the variance of measurement in the population in which it was assessed. Bland Altman plots were a more appropriate method of assessing the degree of agreement. These plot the difference between two methods against the mean of the methods, disclosing the variability, any systematic difference between the means, without assuming that either method has superior properties. Figure 4.6 demonstrated that measurements of fat mass by 4C were higher than those obtained with QMR at week 0 and week 6 but lower at week 16. The regression lines showed no significant slopes but did demonstrate the mean differences (bias) ± SD of 0.94kg±2.56, 0.18±2.7 and -0.67kg±2.69 for week 0, week 6 and week 16 data. The large 95% confident intervals on the plot reflect that either technique was not correct particularly at the time of acute weight loss. Measurement precision, however, remained high.

Figure 4. 5.   Correlation plots comparing fat mass by QMR and 4C model
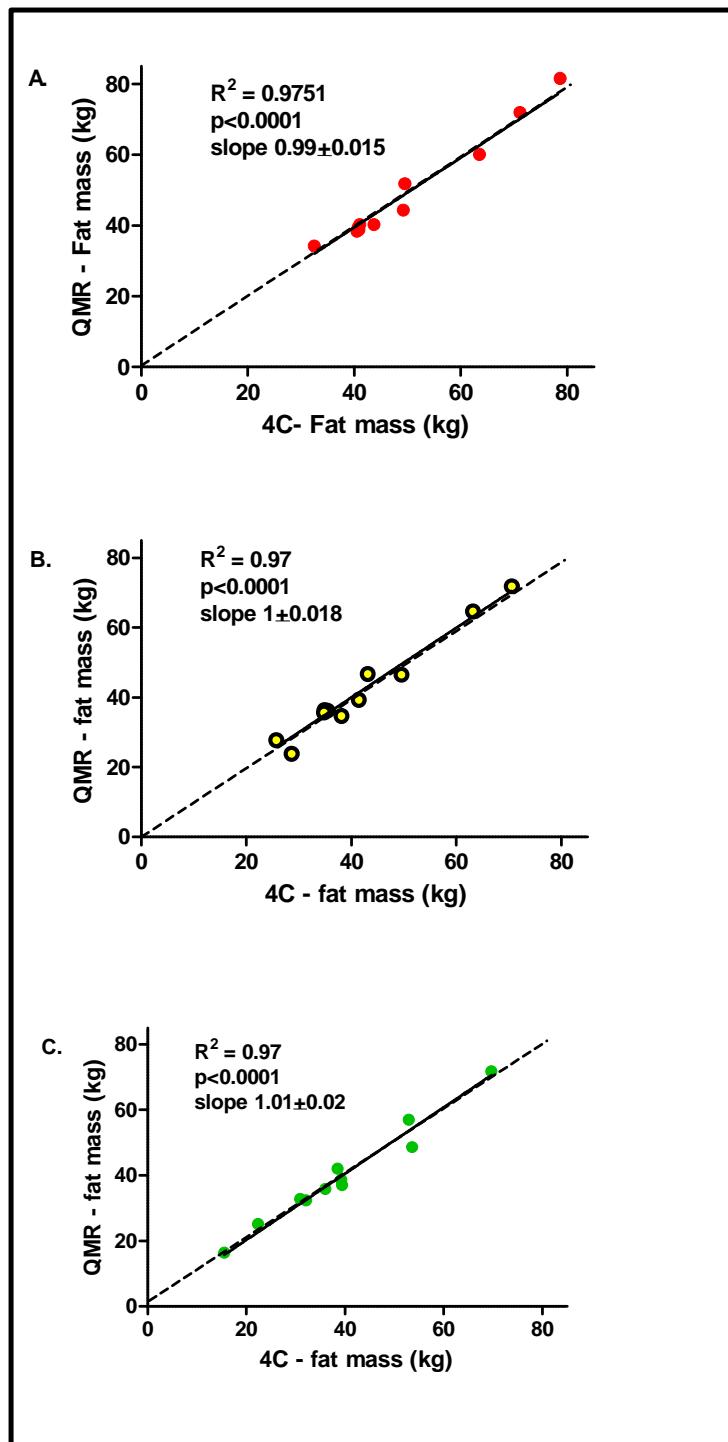
A= baseline fat mass at week 0, B= fat mass at week 6 and C= fat mass at week 16.

The solid line is a linear regression through the data points. The dotted line is the line of identity between the two methods.

Figure 4. 6.   Bland-Altman graph of absolute Fat mass measured by 4C and QMR techniques
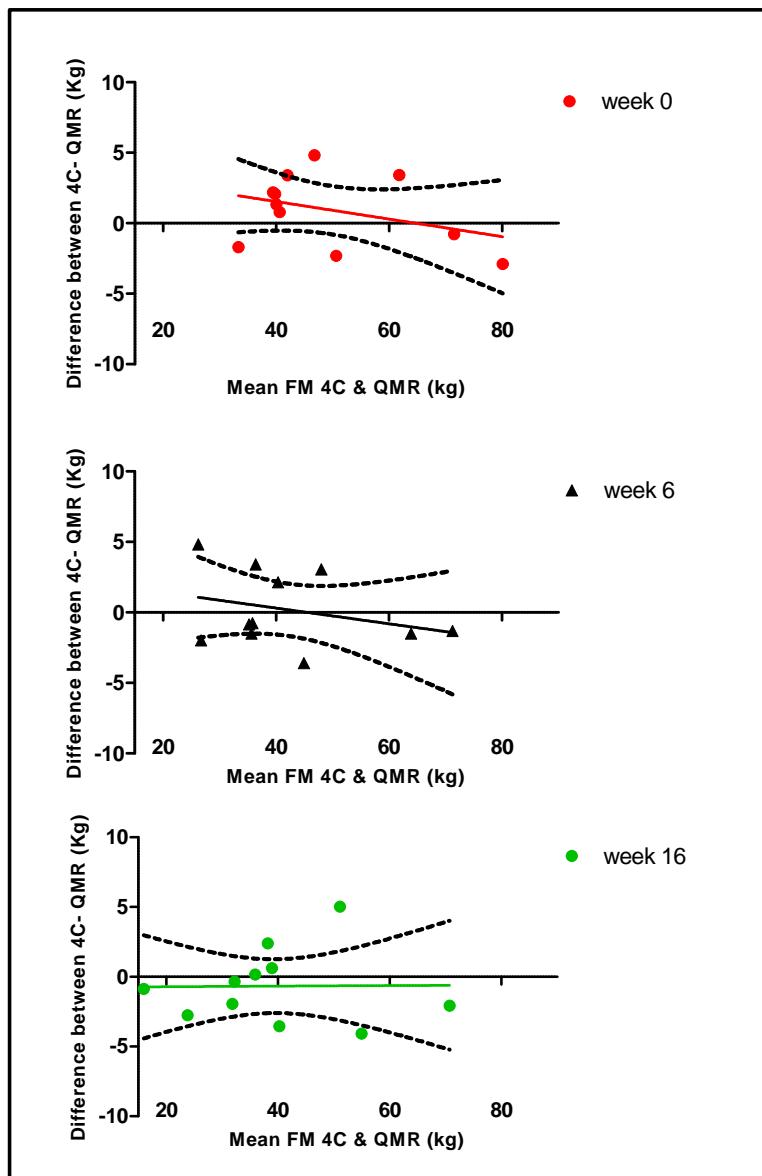
The horizontal line is the line of identity of two methods. A linear regression through the data points is shown with a solid line (red- week 0, black – week 6 and green – week 16), with dashed lines defining the boundaries of the 95% confidence intervals.

Similarly, fat mass loss measured by the different methods showed strong correlation (Figure 4.7). Bland-Altman analysis also showed no significant slope when they were plotted against delta fat mass (fat mass loss) at week 6 and 16 compared with week 0 (Figure 4.8).

Figure 4. 7.   Correlation plots comparing delta fat mass by QMR and 4C model
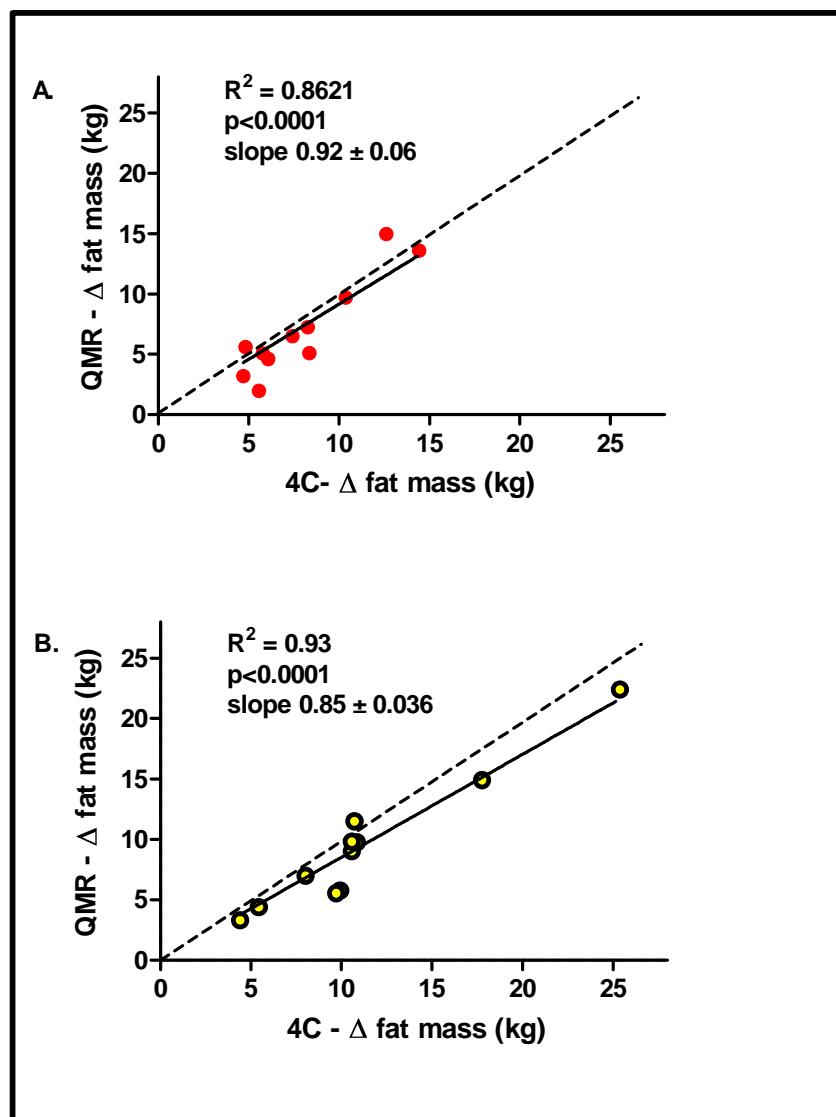
The dotted line is the line of identity of two methods. A linear regression through the data points is shown with a solid line (red- week 6 -0, yellow – week 16 –0).

Figure 4. 8.   Bland-Altman graph of delta Fat mass (FM loss) comparing 4C and QMR techniques
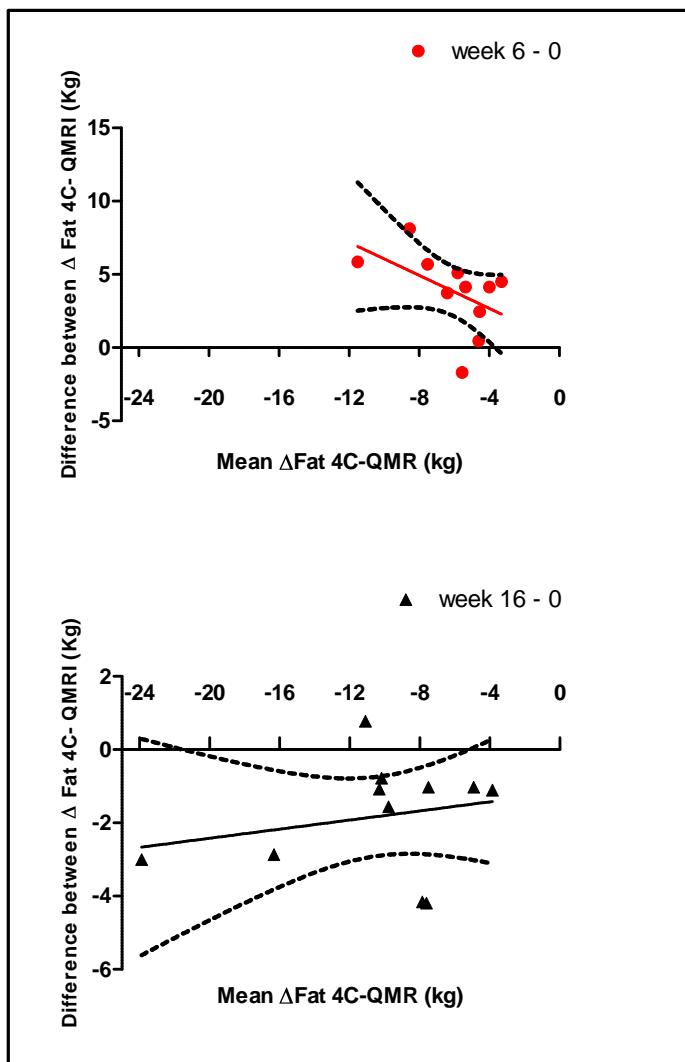
The horizontal line is the line of identity of two methods. A linear regression through the data points is shown with a solid line (red= week 6 -0, black = week 16-0), with dashed lines defining the boundaries of the 95% confidence intervals. At week 6, FM loss from baseline, measured by 4C was higher than QMR. The reverse was seen at week 16.

Three patients did not have DXA at baseline due to their initial weight exceeding the scan's weight limit. Therefore, FM measured by DXA was not used to compare with other techniques. However, DXA was used in the calculation of the 4C model as well as assessing regional fat weight loss. Android FM was contributed by visceral/abdominal FM.

Traditionally, waist and hip circumferences were used to measure the pattern of obesity i.e., android or gynoid obesity (**section 1.1.2**). In the current study, regional FM i.e., FM at android (abdominal/visceral) and gynoid (pelvis) regions measured by DXA were used to assess the pattern of obesity. Changes in android and gynoid regional fat mass ratio (AG ratio) at week 6 and week 16 from baseline were shown in Figure 4.9. AG ratio significantly improved (reduced) from a baseline value of 0.70± 0.19 to 0.65 ±0.15 at week 6 and 0.60±0.16 kg at week 16 following weight loss (p=0.0136).

Figure 4. 9.   Android and gynoid fat mass ratio measured by DXA
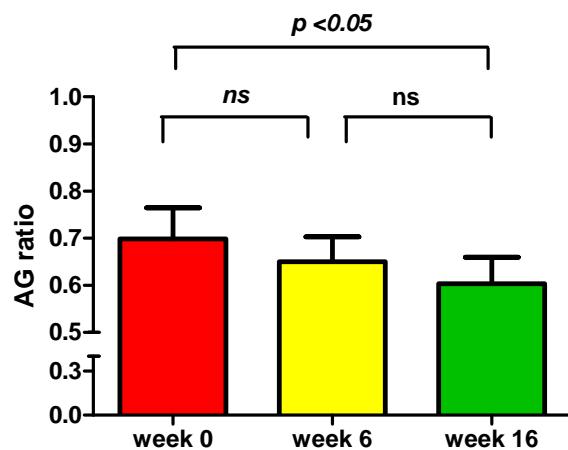
Bar graph representing means and standard error of means. N=8. The omnibus significance for the trend was *p=0.0136.* The significant was determined by 1-way ANOVA repeated measures, and *post hoc* testing with Bonferroni's adjustment.

The reduction in android FM (from week 0 to week 6) was significantly correlated with reduction in total body mass, total body FM and gynoid FM and fasting insulin level. (Figure 4.10)

Figure 4. 10.   Correlation of changes in (android) visceral fat mass measured by DXA (week 16 – week 0) with changes in other body composition measures and fasting plasma insulin level
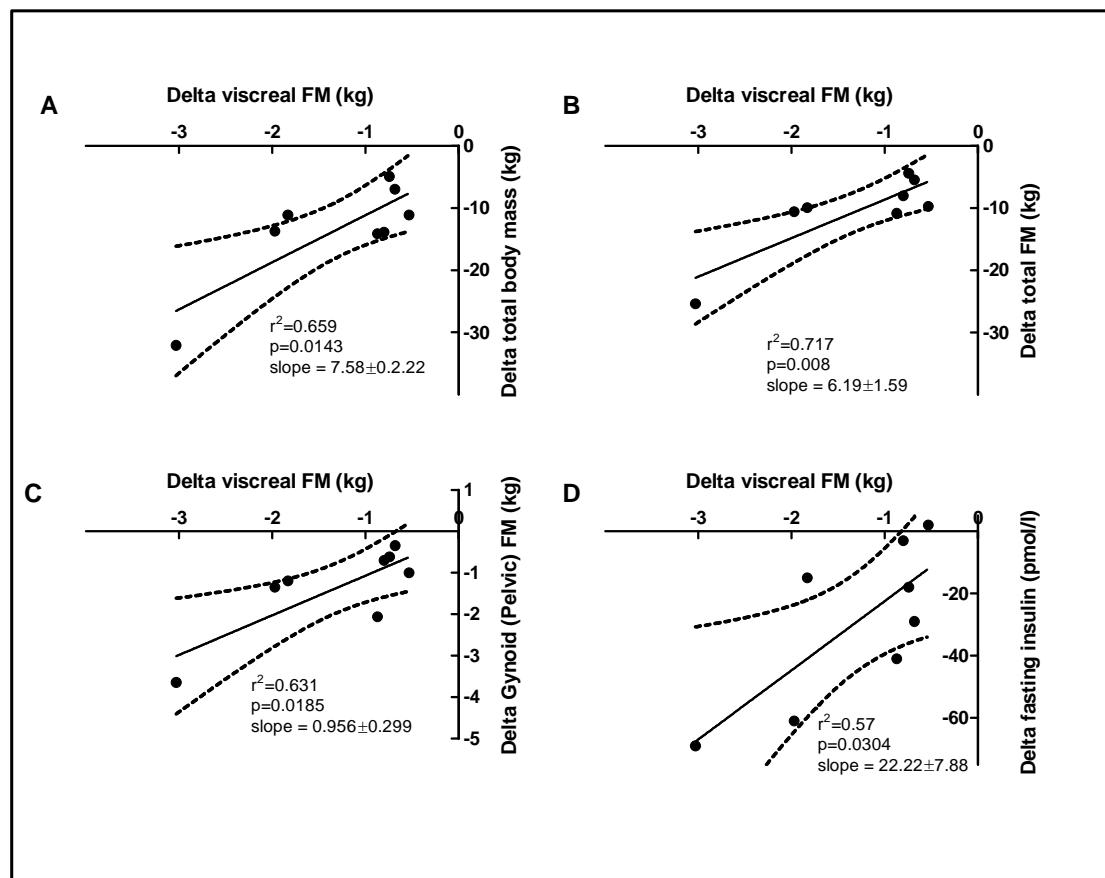
A) total body mass, B) total FM C) gynoid (pelvis) fat mass and D) fasting plasma insulin level

It demonstrated correlations between changes in android (visceral) FM (kg) and other collected variables in patients with obesity and heart failure/ high CVD risk. Correlation and significance was determined using Pearson's Correlation test. Correlation is significant* at the level 0.05.

The correlation between the fat mass measured by 4C and other putative variables are illustrated in Table 4.5. As expected, FM was significantly correlated with waist circumferences and plasma leptin level (correlation coefficient 0.595 and 0.712, p = 0.027 and 0.014 respectively). However, other variables including cardiac performance parameters (peak VO$_2$, anaerobic threshold, LVEF, LV mass), metabolic profile (total cholesterol, HDL, LDL, trigycerides, ALT, fasting glucose, insulin), resting BNP, plasma norepinephrine sensitive CRP, TNFα and total antioxidant profile (FRAP) did not show any significant correlation with fat mass.

Table 4. 5.   Correlations between 4C fat mass and putative variables

Variable	Correlation Coefficient	Significance
Waist circumference	0.595	0.027*
Peak VO$_2$(ml/kg/min)	-0.371	0.13
Anaerobic threshold (ml/kg/min)	-0.35	0.15
LVEF (%)	0.191	0.29
LV mass (g)	0.434	0.19
Resting BNP (pg/ml)	0.147	0.34
Sensitive CRP (ng/l)	-0.24	0.23
Plasma NE (ng/l)	0.339	0.15
Fasting insulin (pmol/l)	-0.158	0.32
ALT (U/l)	0.442	0.09
Total cholesterol (mmol/l)	0.254	0.23
Fasting insulin (pmol/l)	0.263	0.22
Adiponectin (μg/ml)	0.505	0.057
Leptin (μg/l)	0.712	0.014*
TNF-α (pmol/l)	0.069	0.43
TAS-FRAP (μmol/l)	0.147	0.342

Table demonstrates correlations between fat mass (kg) and other collected variables in patients with obesity and heart failure/ high CVD risk. Correlation and significance was determined using Pearson's Correlation test. Correlation is significant* at the level 0.05.

4.6.   Body composition changes - Discussions

The aim for achieving acute weight loss and the weight loss maintenance were met. Both clinically and statistically significant weight loss (mean 10.6%) was achieved at 6 weeks and maintained until the end of the study (mean 12.6%). As expected, loss of fat mass was much greater than loss of lean mass (22.14% Vs 5.23%, p <0.0001). The lean mass loss was greater at week 6 than at week 16 (6.7% Vs 5.2%). This can be explained by the fact that early weight loss following a low calorie diet used glycolysis as an initial fuel source. Kreitzman et al[246] measured total body potassium loss to estimated glycogen loss on the basis that each gram of glycogen is associated with 0.45mmol of potassium loss. In the first 4 days of VLCD, there was an average of 180mmol loss of total body potassium and hence equivalent glycogen depletion of around 400g. Krotkiewski M et al[247] also confirmed the loss of glycogen following VLCD by performing skeletal muscle biopsies. The estimate of glycogen storage in lean subjects in a fed state is generally around 400-500g with associated water of average of 1.6kg (range from 1.2-2kg i.e., three to four times the glycogen weight). This accounts for the initial weight loss of 2-2.5kg following VLCD. Glycogen, as carbohydrate, contributes 4kcal/g whereas fat yields 9kcal/g of energy. Therefore, it requires about 2500kcal deficit to achieve 2.5kg of weight loss by glycolysis (burning glycogen) whereas a 22500kcal deficit will be required to burn fat (lipolysis) [248] (Equation 13 and Equation 14).

 Equation 13.

2.5kg weight loss = 2.5/ 1.6 x 400g glycogen = 625g of glycogen = 625 x 4kcal = 2500kcal deficit

Equation 14.

2.5kg weight loss = 2500g fat = 2500 x 9kcal fat = 22500kcal deficit

If a person's daily energy expenditure is at around 2300 kcal/day and calorie intake of 1000kcal/day, (1300kcal/day energy deficit) it will take him 17.3 days to achieve 2.5 kg

fat loss. Therefore weight loss of 2.5-4kg in a week by restricting energy intake of 1000kcal/day cannot be entirely the fat loss but contributed to glycolysis and fluid loss.

The patterns in body composition changes were encouraging. Though all 4 components have reduced in mass, if changes were illustrated as a percentage of total mass, FM was reduced in contrast to FFM which was increased. In addition, these body composition changes were in parallel to an improvement in their metabolic profile. (see section 4.9).

The changes in regional fat loss assessed by DXA were interesting. Though there were reduction in both android and gynoid FM, a significant reduction in AG fat mass ratio was also demonstrated. The AG ratio has not been used to assess the differential fat mass loss before and it suggested a possible reduction of FM more from the intra abdominal region than that of around the pelvic region. In addition, reduction in android fat ratio was also strongly associated with fasting plasma insulin level which may imply a reduction in degree of insulin resistance. The current study did not measure specific visceral FM differentiating it from subcutaneous abdominal FM. Therefore, future studies to compare the DXA with other imaging modalities such as MRI or proton-magnetic resonance spectroscopy ((1)H-MRS to assess the regional fat mass would have been useful to correlate with other various metabolic changes. DXA scan used in the study was not ideal for morbidly obese patients and newer scanning that accommodates larger body size would have been more desirable. As a consequence, apart from BMC, body composition data (FM, regional FM changes) in three individuals from the current study was not able to be included in the data analysis.

**4.7.   Cardiac performance, haemodynamic changes and sympathetic activity -
Results**

For each cardiopulmonary exercise test, all patients achieved the Borg scale of 17.
Individuals could exercise longer post weight loss (12 ± 5.2, 14.5 ± 5.1, 16 ± 5 min at week
0, 6 and 16 respectively, p=0.0013), achieving a higher Naughton stage (5.7 ± 1.3,
6.5 ± 0.8, 6.7 ± 0.6 p=0.0006). At baseline, peak VO_2 correlated significantly with a
number of other cardiac prognostic indicators. The detailed correlation was shown in
Table 4.6. Left ventricular ejection fraction, left ventricular mass, plasma BNP and
anaerobic threshold significantly correlated with peak VO_2.

Table 4. 6.   Correlations between peak VO_2 and putative variables at baseline

Variable	Correlation Coefficient	Significance
4C Fat mass (kg)	-0.338	0.15
LVEF (%)	0.589	0.028*
LV mass (g)	0.551	0.04*
Anaerobic threshold (ml/kg/min)	0.931	<0.0001*
Resting BNP (pg/ml)	0.610	0.031*
Sensitive CRP (ng/l)	0.041	0.45
Plasma NE (ng/l)	0.162	0.32
Urinary NE (nmol/24h)	-0.311	0.19

Table demonstrates correlations between peak VO_2 and other collected variables in
patients with obesity and heart failure/ high CVD risk. Correlation and significance was
determined using Pearson's Correlation test. Correlation is significant* at the 0.05 level.

The primary end of the study, Peak VO_2 corrected for BW (ml/kg/min) showed 14.34% and 13.26% improvement (18.33±4.5, 20.96±4.8, 20.76±4.8 ml/kg/min, p= 0.0095) (Figure 4.11). There were no significant changes in absolute peak VO_2 (ml/min), peak VO_2 corrected for FFM (ml/fat free mass kg/min), anaerobic threshold and VE/VCO_2 slope.

Figure 4. 11.   **Peak VO_2 changes (adjusted for body mass)**
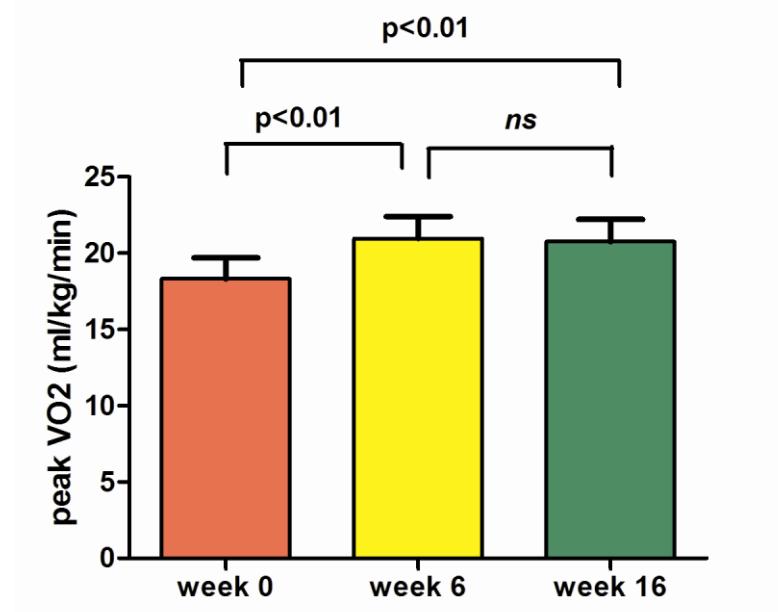
Bar graph represents means and standard error of means. N=11. The omnibus significance for the trend was *p=0.0095*. The significant was determined by 1-way ANOVA repeated measures, and *post hoc* testing with Bonferroni's adjustment.

The effect of WL and WLM on cardiac performance, haemodynamic changes and sympathetic activity are demonstrated in Table 4.7. Echocardiogram did not show any significant changes in LV ejection fraction, LV mass, interventricular septal thickness or posterior wall thickness. Measurement of right ventricular systolic pressure and systolic pulmonary arterial pressure was not available in some cases due to the lack of significant regurgitant flow across the valve. Therefore analysis was not carried out for those measurements.

Table 4. 7. **Effect of weight loss and weight loss maintenance on cardiac performance, haemodynamic changes and sympathetic activity**

	baseline	Week 6	Week 16	P value
Peak VO$_2$ (ml/kg/min)$^+$	18.33±4.5	20.96±4.8*	20.76±4.83*	0.0095
Peak VO$_2$ (ml/min)$^{++}$	2107±605	2083±596	2054±471	0.8
Peak VO$_2$ (ml/ FFM kg/min)$^{+++}$	33.74±6.6	35.84±8.3	34.82±5.5	0.4
AT (ml/kg/min)	12.08±2.5	13.89±3.2	13.63±3.2	0.07
VE/VCO$_2$ slope	27.61±5.2	28.36±6.9	26.74±5.7	0.2
LVEF (%)	49±13.2	49.73±15	52.45±14.6	0.08
LV mass (g)	296.5±74.7	264.5±83.9	271.5±78.2	0.057
IVS thickness (cm)	1.24±0.08	1.16±0.17	1.17±0.09	0.2
Posterior wall thickness (cm)	1.14±0.16	1.03±0.14	1.08±0.1	0.3
Systolic BP (mmHg)	142.7±19	120.5±15*	131.5±14	0.0008
Diastolic BP (mmHg)	81.5±9.9	74.2±13.6	80.2±8.8	0.1
Resting heart rate (bpm)	76.1±16.9	65±12.7*	65.5±14.7*	0.007
Plasma NE (ng/l)	435±142	328.4±134*	318.5±124*	0.03
24h urinary NE (nmol/24h)	397±255	227±105*	297±153	0.009
Plasma BNP (pg/ml)	74.09±109	55.53±95	72.73±109	0.2037
LF power(ms^2)	66.94±65.8	103.9±97.12	149.7±102.3*	0.0036
HF (ms^2)	67.08±67.3	94.67±123	246±291*	0.0334
SDNN (ms)	34.46±20	40.7±17.5	49.15±22.1	0.1
SMSSD (ms)	32.7±30	27.3±16.8	34±18.4	0.6

Values represent mean ± standard deviation. n=11. Significance was determined by one way ANOVA with repeated measure. When the omnibus test was significant (final column), Bonferroni multiple comparisons was performed for baseline *versus* week 6, baseline versus week 16. Significant difference is indicated by * (p <0.01). There was no significant difference between week 6 and week 16 for any of the parameters.

Heart rate variability parameters: HF (high frequency), LF (low frequency), SDNN (Standard deviation of the NN interval, SMSSD (Square root of the mean squared differences of successive NN interval)

[+] indicates value corrected by body mass whereas [++] was absolute value and [+++] being values corrected by fat free mass, NE (norepinephrine).

The systolic BP and heart rate reduced significantly (142.7±0.16 to 120.5±15 and 131.5±14 mmHg, p= 0.0008) (Figure 4.12) but there was no change in diastolic blood pressure. The degree of fall in BP likely to be more significant as four out of five patients (80%) who were on antihypertensive therapy needed to reduce their medication during WL to prevent hypotension.

Figure 4. 12.   Systolic blood pressure and heart rate changes
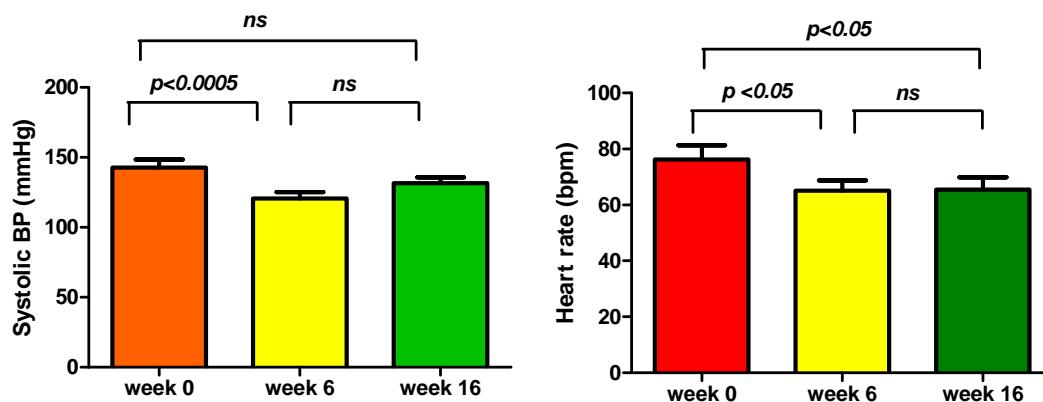
Bar graph represents means and standard error of means. N=11. The omnibus significance for the trend was *p=0.0008* for systolic BP and *0.007* for heart rate. The significant was determined by 1-way ANOVA repeated measures, and *post hoc* testing with Bonferroni's adjustment.

In terms of changes in autonomic activities, there were significant reductions in resting heart rate (76.1±16.9 to 65±12.7 and 65±14.7bpm, p=0.007) (Figure 4.12), plasma norepinephrine (435±142 to 328.4±134 and 318.5±124 ng/l, p= 0.03) and 24 hour urinary norepinephrine level (397±255 to 227±105 and 297±153, p=0.009) from baseline to week 6 and week 16 respectively (Figure 4.13). Heart rate variability measurements demonstrated significant increases in both low frequency (LF) and high frequency power (HF) in the frequency domain but no significant changes were noted in LF/HF ratio. Time domain assessment did not show any significant changes in SDNN

and SMSSD. Overall it can be concluded that there was an improvement in sympathetic activity.

Figure 4. 13.   Changes in resting plasma and urine norepinephrine level
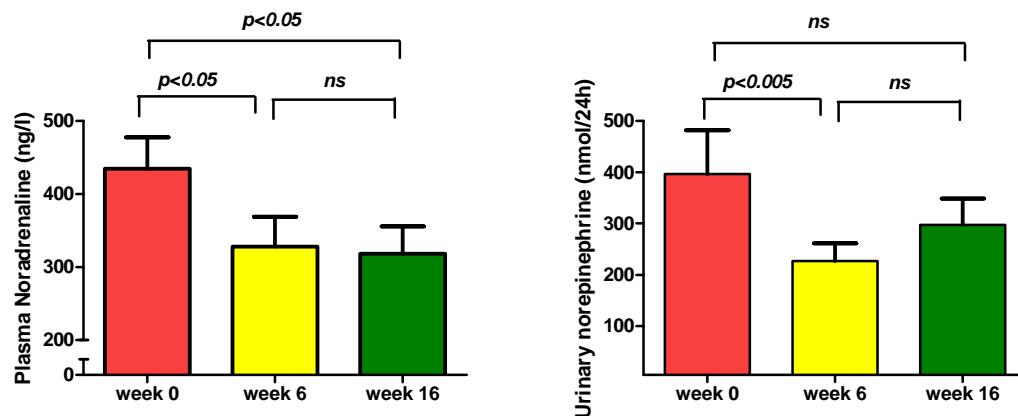
Bar graph represents means and standard error of means. N=11. The omnibus significance for the trend was *p=0.0311* for plasma and *p=0.0094* for urine norepinephrine. The significant was determined by 1-way ANOVA repeated measures, and *post hoc* testing with Bonferroni's adjustment.

Figure 4. 14. Changes in high frequency and low frequency power (heart rate variability)
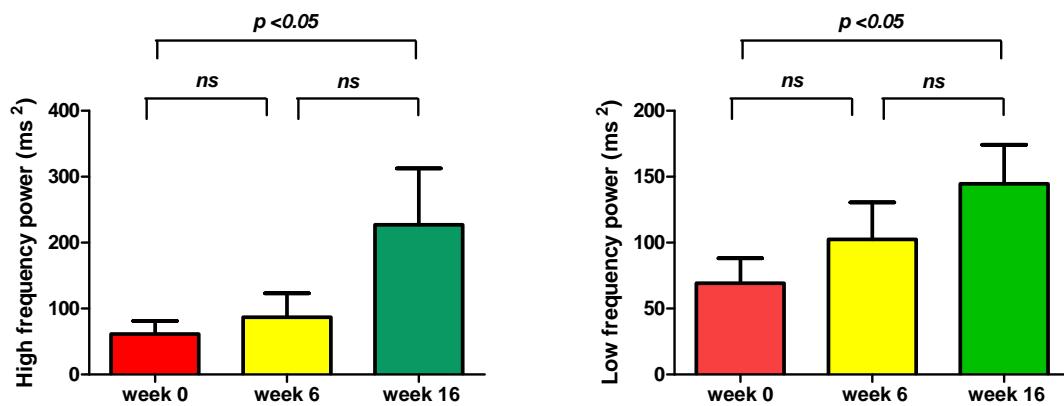
Bar graph represents means and standard error of means. (N=11) The omnibus significance for the trend was *p=0.0334* for high frequency power and *p=0.0036* for low frequency power. The significant was determined by 1-way ANOVA repeated measures, and *post hoc* testing with Bonferroni's adjustment.

The correlation of fat mass with other various parameters of sympathetic activity at baseline was shown on (Table 4.8) These included heart rate, systolic blood pressure, heart rate variability (HF, LF, LF/HF ratio, RSMDD, SDNN) and plasma norepinephrine. There was no significant correlation seen in any of those parameters. The lack of association could be explained by the influence of other factors on sympathetic activities such as therapy with antihypertensive medication, the heterogeneity of their underlying cardiac status (NYHA status of heart failure from normal to stage 3) and presence or absence of diabetes.

Table 4. 8.   Correlations between Fat Mass measured by 4C and putative variables of sympathetic activities at baseline

Variable	Correlation Coefficient	Significance
Heart rate	-0.013	0.97
Systolic BP	0.235	0.49
Diastolic BP	-0.216	0.52
High frequency power	0.076	0.82
Low frequency power	0.053	0.88
LF/HF ratio	-0.204	0.55
SDNN	-0.119	0.73
RMSSD	-0.249	0.46
Plasma NE	-0.239	0.48

Table demonstrates correlations between Fat mass and other collected variables of autonomic function (sympathetic activity) in patients with obesity and heart failure/ high CVD risk. Correlation and significance was determined using Pearson's Correlation test. Correlation is significant* at the level 0.05. None of the parameters of sympathetic activity was significantly correlated with fat mass at baseline.

Figure 4.5 showed the inverse correlation between LVEF and the resting plasma BNP level and log plasma BNP level indicating known relationship of BNP as a marker of left ventricular function. However, there were no significant correlations between changes in BNP at week 16 to baseline (delta BNP) with changes in other collective parameters including LVEF, body composition measurement, peak VO2, plasma insulin, plasma norepinephrine and heart rate variability. (Table 4.9)

Figure 4. 15. Correlation between left ventricular ejection fraction and plasma BNP
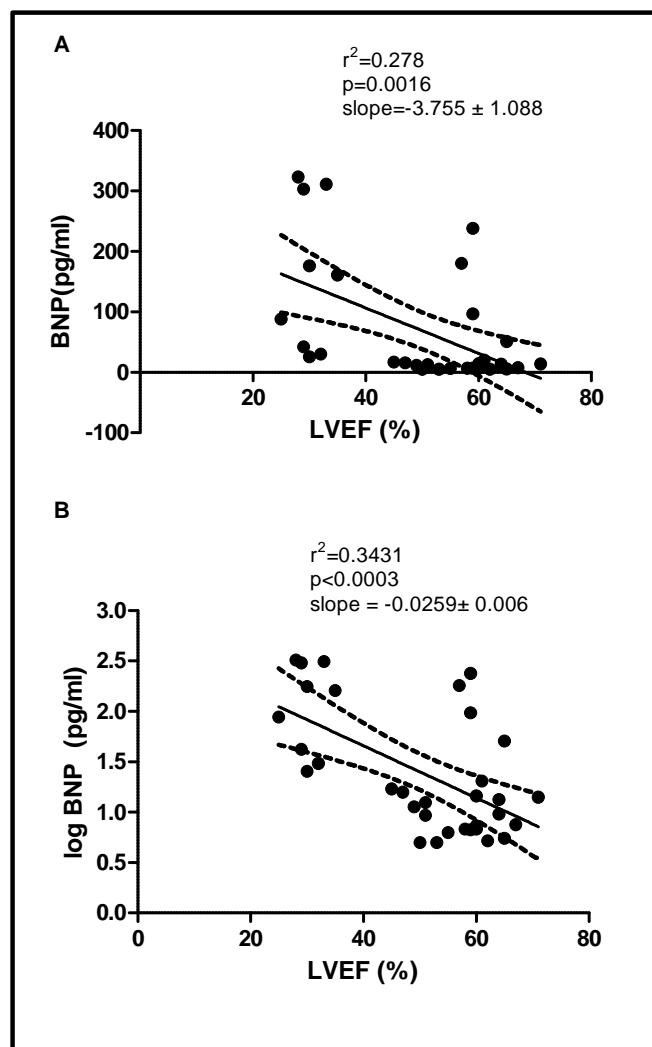
Left ventricular ejection fraction (LVEF) correlated with plasma BNP in obese patients with heart failure and or high cardiovascular risk. **A** represents correlation between BNP and LVEF whereas **B** represents those of log BNP and LVEF. Both graphs demonstrated significantly higher BNP and log BNP levels as LVEF levels become lower.

Table 4. 9.   Correlations between changes in resting plasma BNP at week 16 from baseline (delta BNP) and putative variables

Variable	Correlation Coefficient	Significance
Delta weight (kg)	0.071	0.84
Delta 4C Fat mass (kg)	0.008	0.98
Delta 4C Fat Free Mass (kg)	0.19	0.58
Delta LVEF (%)	0.05	0.88
Delta Peak VO$_2$	0.007	0.98
Delta Plasma insulin (pmol/l)	0.311	0.35
Delta Plasma NE (ng/l)	-0.248	0.463
Delta HF (heart rate variability)	-0.062	0.86
Delta LF (heart rate variability)	-0.41	0.21

Table demonstrates correlations between plasma BNP changes from baseline to 16 weeks and other collected variables in patients with obesity and heart failure/ high CVD risk. Correlation and significance were determined using Pearson's Correlation test. Correlation is significant* at the 0.05 level.

4.8. Cardiac performance, haemodynamic changes and sympathetic activity - Discussions

All patients tolerated the cardiopulmonary exercise testing well. With weight loss, the endurance of individuals improved because the exercise time taken to a similar Borg scale took longer to reach and they achieved a higher Naughton stage. Peak VO_2 is generally reported after correction for patient body mass (ml/kg/min) or as an absolute value (L/min). It is debatable as to how to report pVO_2 in people who lost weight. Some suggest pVO_2 in reference to the lean body mass (FFM) would be a better index because fat metabolism does not contribute much to VO_2.[249, 250] So far, there is no consensus conclusion for how to report pVO_2 as yet. In this study, when pVO2 was reported as a standard way i.e., corrected for BW (ml/kg/min), [110, 114, 115] an improvement was seen. This is intuitively 'correct' i.e. it represents the fact that they could exercise more. The absolute peak VO_2 (ml/min) and that corrected for FFM (ml/lean mass kg/min) were however not affected by weight loss. This could also imply no deterioration of pVO_2 by weight loss. On the other hands, there were no significant changes in other prognostic markers of cardiopulmonary exercise, anaerobic threshold and VE/VCO_2 slope.

Other than the mechanism of VO_2/kg effect, it was possible that weight loss improved cardiac performance by reduction in intramyocellular lipid and/or selective reduction of epicardial fat. Increase epicardial fat is common in morbidly obese individuals and it has been linked to visceral adiposity and insulin resistant.[251, 252] MRI spectroscopy has been used to quantify triglyceride content in human myocardium (intramyocellular triglyceride) and found it to be significantly increased in obese compared with normal-weight subjects.[251] Weight loss following VLCD in obese patients with type 2 diabetes significantly decreased myocardial triglyceride level, reduce left ventricular mass and improved LV diastolic function.[253] Improved LV diastolic function and decreased in LV mass was demonstrated by average reduction of epicardial fat thickness of 4mm with VLCD of 6 months.[254] Therefore, measurement of both total body fat mass and the intramyocellular triglyceride would have been useful to provide further information.

Overall, it would be reasonable to conclude that the peak VO_2 should still be a primary end point because it represented an overall functional status i.e., cardiac performance.

Echocardiography did not show any significant changes in LV ejection fraction, LV mass, interventricular septal thickness or posterior wall thickness. This was predicted due to the relatively short duration of the study and hence, LVEF was not chosen as a primary outcome. However, the trend in LVEF increased with a decreased in LV mass and IVS thickness. This might suggest an improvement in overall cardiac function. Echocardiography was technically difficult in obese individuals and could provide poor image quality. Measurement of cardiac parameters by Cardiac MRI was intended before the study was conducted. Unfortunately, the machine was yet not available when the study was conducted. On the other hand, three of our cohort would not fit in to the proposed MRI machine at baseline because their waist circumferences would have exceeded the upper limit of the machine.

Despite discontinuation of antihypertensive therapy, systolic BP reduced significantly by an average of 22.18 and 11 mmHg at 6 weeks and 16 weeks respectively compared with the baseline. This could be explained by understanding the mechanism of hypertension in obesity. The reduction of systolic BP was thought to be related to changes in cardiac output (CO) and systemic peripheral resistance (SVR) (BP = CO x SVR). As described, obesity is associated with haemodynamic changes as well as increased peripheral vascular resistance by enhancing endothelial dysfunction, insulin resistance, and stimulation of the sympathetic nervous system and release of adipokines (session 1.2). Reduced renal sodium excretion, volume expansion and sympathetic nervous system stimulation are recognised features of changes seen in obese individuals.[255] Hyperinsulinaemia enhances renal sodium retention not only directly, through its effects on renal tubules,[256] but also indirectly, through stimulation of the sympathetic nervous system[257] and through increased angiotensin II-mediated aldosterone secretion.[258]

In this study, we have used a fixed amount of diet (800kcal/day for women with BMI<40 and 1000kcals/day for all men and women with higher BMI) in the initial 6 weeks of a low energy liquid diet phase. It was likely that the salt content of this regimen was lower than the subjects' previous intake. In addition, as previously stated, in the initial phase of acute weight loss, diuresis occurred as patients lost a significant amount of glycogen and water.[259, 260] This resulted in a relative intravascular fluid loss, reduced peripheral resistance and subsequent reduction in blood pressure. In the steady state of weight maintenance, individuals regained 50% of weight they lost during the WL phase. The degree of overall systolic BP reduction during WLM phase is therefore more likely as a consequence of weight loss. This was mediated by reductions in resting heart rates, improvements in sympathetic nervous system function (reduced noradrenaline level, improved heart rate variability) as well as reductions in fasting insulin levels indicating a degree of improvement in insulin resistance. The degree of reduction in systolic BP can be compared with the UK Prospective Diabetes Study where 10 mmHg reduction of mean systolic BP was associated with a reduction in 12% of any diabetes related complication, 15% of deaths related to diabetes, 11% of myocardial infarction and 13% of microvascular complications.[114, 261]

The measures of sympathetic activity (resting heart rate, resting plasma norepineprine and heart rate variability) showed significant improvements. The improvement in HRV was mainly seen in the frequency domain i.e., increased high frequency power and low frequency power. Parasympathetic activity contributes both components whereas sympathetic activity mainly contributes to LF. The reducing trend in the LF/HF ratio although not significantly, confirmed the contribution of LF was likely from the parasympathetic component. The time domain parameters did not significantly change. In general, longer recording for over 24 hours usually provides better information with regards to time domain measurement. Unfortunately, it was not able to perform in the current study because the recording tape had to come of for various body composition measurements and patients would have required staying one more night to achieve this.

In addition, it was predicted that 15 min recording would also give reasonable information of HRV.

Sympathetic system over-activation in this study group was contributed by impact of their body mass as well as their underlying cardiac status. As stated detail in section 1.3.6, there is a significant association between sympathetic over-activation and cardiovascular mortality. Obesity has an impact on cardiac autonomic function. A 10% increase in body weight is associated with a decline in parasympathetic tone and increased sympathetic activity, accompanied by a raised heart rate.[262] Conversely, heart rate declines during weight reduction.[262] Several studies have shown that obesity is associated with reduced heart rate variability (HRV) and hence impairment of autonomic function in obesity.[263-265] Kim et al[263] have reported a high level of Fat mass was associated with lower level of LF (r = -0.34) and lower level of root mean square difference of successive NN interval (RMSSD) (r=-0.33). Sympathetic activation is also associated with hypertension,[266] hyperinsulinaemia[257] and type 2 diabetes.[266] In experimental rats, calorie restriction induced a decrease in norepinephrine (NE) turn-over rate, i.e., reduced sympathetic activity in contrast to overfeeding which stimulate SNS activity.[267] A 10% weight loss in severely obese patients is associated with significant improvement in autonomic nervous system cardiac modulation (decreased heart rate and increase HRV).[265] The present study confirmed the finding of an improvement in HRV after achieving 12% weight loss. Similarly, Karason et al[268] has demonstrated that following a mean of 28% weight loss in obese patients after weight-reducing gastroplasty, there were improvements in autonomic functions (reduction blood pressure and norepinephrine excretion and increments in SDANN and HF values).

4.9.   Metabolic profile and biochemistry - Results

The detail changes in metabolic profile and biochemistry were illustrated in Table 4.10. With regards to glycaemia, all insulin treated patients achieved at least a 60% reduction in their insulin requirement. One patient managed to come off insulin all together and subsequently she only needed metformin. In those who were not treated with insulin, fasting insulin levels were significantly reduced (83.6±44.3 to 52.9±32 and 56.2±48.1 pmol/l, p=0.0117). Among all the patients, glycaemic control generally improved and two patients remained euglycaemic despite being no glucose lowering agent. There was a significant improvement in HbA1c at the end of 16 weeks (from baseline of 7.22±1.4 to 6.35±1.83% at week 16). Though there was a trend in lower fasting glucose levels, it was not statistically significant.

Total cholesterol levels were significantly reduced during acute weight loss phase (4.18±0.6 to 3.47±0.06 mmol/l), but increased during WLM (3.93±0.7mmol/l). The initial reduction was due to the statistically and clinically significant reduction in triglyceride levels (1.9±0.93 to 1.2±0.62 mmol/l) and a trend toward lower levels of HDL. TG continued to fall significantly during WLM phase to 1.29±0.49 mmol/l whereas HDL increased (not statistically significant). LDL cholesterol, however slightly increased during WLM phase (2.35±0.6 to 2.43±0.7 mmol/l) from baseline towards the end of the study.

Significant improvements in other metabolic parameters i.e., ALT, TNF-α and CRP were also seen. There was no change in adiponectin and IL-6 levels. Unfortunately, there were no report for 3 IL-6 samples because there were some errors in the laboratory and therefore reports of these individual were not included in the analysis. The analysis was therefore based upon 8 individuals. As expected, leptin levels were reduced during the acute weight loss phase. The levels then increased during WLM or re-feeding phase but were still lower than baseline. Total antioxidant status (TAS) measured by FRAP

assay was significantly reduced. (From baseline of 886±258 to 760±221 and 689±178 μmol/l, p=0.0009).

Table 4. 10   Effect of weight loss and weight loss maintenance on metabolic profile and other biochemistry

	baseline	Week 6	Week 16	P value
Fasting glucose (mmol/l)	7.44±2.66	6.72±2.68	6.35±1.83	0.1
Fasting insulin[#] (pmol/l)	83.6±44.3	52.9±32*	56.2±48.1	0.2
HbA1c (%)	7.22±1.4	6.6±1.2	6.3±1.5*	0.0117
Adiponectin (μg/ml)	4.62±2.3	3.85±1.9	4.51±2.5	0.4
Leptin (μg/l)	52.87±4.9	18.2±12.8*	37.47±25.3	0.0126
ALT (U/l)	41±20.8	35.8±15.5	26.8±8.2*	0.0368
Total cholesterol (mmol/l)	4.18±0.6	3.47±0.6*	3.93±0.7[+]	0.0005
LDL (mmol/l)	2.35±0.6	2.07±0.6	2.42±0.7[+]	0.0155
HDL (mmol/l)	1.01±0.24	0.92±0.17	1.05±0.23	0.053
TG (mmol/l)	1.90±0.93	1.2±0.62*	1.29±0.49*	0.0037
Sensitive CRP (ng/l)	4.34±3.15	2.81±2.61**	3.04±2.77*	0.0024
TNF-α (pmol/l)	3.87±1.7	3.46±1.6	2.74±2.2*	0.0159
IL-6 (pmol/l)[o]	1.109±1.51	1.003±1.29	1.27±1.3	0.7787
TAS-FRAP (μmol/l)	886±258	760±221*	689±178*	0.0009

Values represent mean ± standard deviation. N=11. Significance was determined by one way ANOVA with repeated measure. When the omnibus test was significant (final column), Bonferroni multiple comparisons were performed for baseline *versus* week 6, baseline *versus* week 16 and week 6 *versus* week 16. Significant difference is indicated by * for first two comparisons and by [+] for the significance difference between week 6 *versus* week 16($p<0.05$). IL-6 levels[o] were based on N=8.

4.10. Metabolic profile and biochemistry – Discussions

With weight loss, glycaemia undoubtedly improved in patients with diabetes. Being a long term marker, HbA1c improvement was only seen in the latter phase of the study. The fall in fasting insulin level together with reduction in requirement of insulin, and other glucose lowering therapy also indicated better glycaemic control and reduced insulin resistance. Initially, HOMA2 model was intended to use to assess insulin resistance. However, three patients from our cohort were treated with insulin and further two patients had insulin levels lower than 20pmol/l at week 16 and therefore inappropriate for HOMA analysis. HOMA2 analysis required insulin level range between 20 to 400pmol/l.[269]

Better glycaemic control was the likely reason why fasting triglyceride fell during acute weight loss and WLM. The trend of transient HDL fall was usually seen in patient undergoing intensive dieting. The underlying mechanism was not entirely clear. Serum ALT, an indicator of non alcoholic hepatic liver disease, was also significantly reduced. There has been concerned of weight loss in patients with hepatic steatosis previously because of the rising trend in ALT.[270, 271] However, growing evidence suggested that increased ALT was transient following weight loss and it would fall down to baseline or lower in long term. Gasteyger et al[272] has showed that weight loss induced by low calorie diet in obese individuals showed immediate reduction in ALT in men and transient small elevation of ALT in women which returned to baseline at the 32 weeks. In the current study, only one male patient had a transient rise (1.5 fold upper limit of normal) was seen but it was then reduced below the baseline at week 16. All remaining patients had a reduction in ALT at both week 6 and week 16.

Significant improvements in other markers of systemic inflammation i.e, TNF-α and hsCRP were also demonstrated with weight loss. Evidence showed that CRP was an independent predictor of cardiovascular disease.[273-275] CRP level was strongly correlated with insulin resistance.[276, 277] In type 2 diabetes, CRP level had a strong relationship

with degree of total and abdominal adiposity, blood pressure and apolipoprotein and triglyceride, independent of genetic influence.[278] The association was probably explained by the fact that IL-6, secreted by adipocytes (an adipokine) was known to be potent stimulant of CRP secretion from liver.[279]

A significant reduction in the CRP level in the current study therefore was likely be contributed by reduction in fat mass. However reduction in total FM and abdominal FM (android) was not significantly correlated to reduction in CRP.

There was no change in adiponectin level. Low levels of adiponectin are found in obese individuals[280] and those with type 2 diabetes.[281] Previously, decreased secretion of adiponectin in the setting of intra-abdominal adiposity suggested an increased cardiovascular risk. However, recent studies suggested that it was not a strong prognostic indicators for CVD risk.[282, 283] In addition, emerging evidences showed a reduced adiponectin was correlated with reduced survival in patients with high CVD risk or established CVD patients. [284, 285] The underlying mechanism was not clear. Therefore, at present, adiponectin is probably not a reliable marker as a monitor of disease progression in established CVD patients.

Being a satiety hormone, leptin level decreased during the acute weight loss phase due to the low energy intake (relative starvation). The level then increased during the WLM or re-feeding phase but still lower than baseline. Reduction in leptin level was predictable following reduction in fat mass. Overall, it can be concluded that the components of metabolic syndrome and adipokine levels improved with acute weight loss and WLM.

Interestingly, total antioxidant status (TAS) measured by the FRAP assay was significantly reduced. The exact reason was not entirely clear. TAS may have decreased due to a lack or a reduction in antioxidant levels in the diet compared to baseline levels

as we did not provide any extra vegetable or fruit during acute weight loss. Crujeiras et al[286] showed a trend for an increase in TAS following weight loss with high fruit intake and a lack of change in TAS following weight loss and low fruit intake. In addition, uric acid level could influence TAS. The present study was also confounded by the fact that all our patients were on vitamins and minerals in WL phase. Oxidant stress is dependent on not only the level of antioxidants but also free radical production. We did not measure lipid hydroperoxide which would probably assess the degree of oxidant stress.[287]

As stated on section 3.9.4, our current study measured TAS by the using FRAP assay. The assay was simple, speedy, inexpensive, and robust and does not require specialized equipment. However, because it relied on the reducing ability of Fe^{+++} to Fe^{++} and the redox potential of Fe^{+++}/Fe^{++} is 0.77 V, any compound having redox potential lower than this could reduce Fe^{+++} to Fe^{++} resulting in a false and high FRAP value.[288] The assay was also based on the hypothesis that the redox reactions proceed rapidly (4 min). However, some antioxidant compounds such as polyphenols (e.g., ascorbic acid) reduce Fe^{+++} very slowly and the reaction does not reach steady state even after several hours of reaction[289]. In addition, the assay does not measure thiol antioxidants, such as glutathione.[290] Some compounds such as bilirubin could give a false high FRAP value because its oxidized product beliverdin absorbs strongly at 593 nm, maximum absorption of ferrous-tridyltriazine complex[291] In the current study, we measured bilirubin level for a safety parameter and no significant changes in the level was noted.

There are many other methods for in vitro determination of antioxidant capacity. They differ from each other in terms of mechanisms of reaction, oxidant and target/probe species, reaction conditions, and in the form that results are expressed.[292] The most commonly used methods were

a) Assays based on biological oxidants
   - peroxyl radical
   - superoxide radical anion

- o hydrogen peroxide
- o hydroxyl radical
- o hypochlorous acid
- o singlet oxygen
- o nitric oxide radical, and
- o peroxynitrite

b) Non-biological assays

- o scavenging of 2,2_-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation (TEAC assay)
- o scavenging of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH• assay)
- o Folin–Ciocalteu reducing capacity (FC assay)
- o electrochemical total reducing capacity

Various methods show various aspects of TAS and each of them have their own pitfalls.[293] In order to show a complete TAS, therefore, would require performing many of those assays. In the current study, assessing TAS was not the main aim. In addition, we did not have the capacity to perform many of those assays. Therefore the FRAP assay which was automated and high through put, was chosen and we believed that it would provide a putative index of anti-oxidant capacity. In retrospect, the detail history of dietary intake i.e, different anti-oxidant capacity of their diet that might impact on the FRAP assay should have been taken. On the other hand, limiting the diet in patients who were already restricted their calorie intake would have been difficult and might have provoked drop out from the study.

Log transformed BNP correlated significantly with LVEF at baseline in line with the literature. However, changes in BNP level were not statistically significant. There was a trend of reduction in BNP at the end of 6th week but it had risen again at 16th weeks almost to the baseline. Evidence showed that the BNP level was inversely proportionate to BMI.[131] The possible explanation was either due to reduction in BNP production or increased clearance of BNP from circulation. The clearance of BNP was mediated by

expression of the natriuretic peptide clearance receptor (NPR-C) in adipose tissue.[294] Subsequently Das et al[131] demonstrated that BMI was also inversely associated with aminoterminal -proBNP (NT-proBNP). NT-proBNP was not believed to bind NPR-C.[295] The authors therefore have concluded that increased clearance was an unlikely mechanism of such an association. They also demonstrated that lean mass measured by DXA, not the fat mass, was associated with low BNP and NT-proBNP. More recently, Chainani-Wu et al[296] conducted a prospective study of 125 patients with either established coronary heart disease or high cardiovascular risk for life style intervention. It was observed that the mean BNP level increased from 18 to 28 pg/ml with a mean reduction in BMI by $2.5kg/m^2$. The percent changes in BNP were inversely associated with the percent changes in BMI. Therefore, the authors have suggested using BNP as a marker of disease progression needed to take into account weight changes. Unlike Chainani-Wu et al, our study did not show a correlation with changes in BNP with changes in fat mass, fat free mass, insulin or catecholamine. We have used a gold standard body composition measurement and other biomarkers. But our cohort had a large spectrum of diseases from high CVD risk to established heart failure patients. The heart failure patients had a higher BNP at baseline. In addition, our cohort was likely to be too small to show a statistically significant difference.

4.11.   Exploratory analysis of changes in fat mass and fat free mass – results

Further analysis was carried out to investigate correlations between changes in FM and the changes in other body composition measures, pVO_2 , cardiac function (LVEF and LV mass), parameters for sympathetic activity (plasma NE and HRV), metabolic parameters (fasting cholesterol, insulin, glucose and CRP). (Table 4.11) As predicted, a FM loss was closely and significantly correlated with the degree of weight loss (r= 0.912, p<0.0001) but not with FFM.

Interestingly, FM loss with significantly and positively correlated with changes in absolute pVO_2 (ml/min) (r=0.793, p=0.004) and pVO_2 corrected by lean mass (ml/FFM kg/min) (r=0.645, p=0.032) but not with pVO_2 corrected by body mass (ml/kg/min) (Figure 4.16). There was no significant correlation with other parameters.

Similar correlation of FFM changes and various parameters were demonstrated in Table 4.12. Again, the FFM loss was significantly associated with the degree of weight loss but not as strong as the correlation between the FM loss and the weight loss (Pearson r of 0.912 *Vs* 0.637, p of <0.0001 and 0.035 respectively). An interesting and negative correlation was noted in between the FFM loss and the pVO2 corrected by body mass (ml/kg/min) (r= -0.688, p=0.019) (Figure 4.17). The correlation was not significant when pVO_2 was corrected with FFM or presented as an absolute value. Similarly, the FFM loss showed no significant correlation was noted with changes in other parameters

Table 4. 11.   Correlation of changes in FM (week 16 – 0) with other putative variables

Parameter	Pearson r	95% confidence interval	P value (two-tailed)	R squared
delta weight (kg)	0.912	0.689 to 0.977	<0.0001***	0.832
delta FFM (kg)	0.281	-0.384 to 0.753	0.404	0.079
delta pVO$_2$ (ml/kg/min)	0.079	-0.547 to 0.648	0.818	0.006
delta pVO$_2$ (ml/min)	0.793	0.369 to 0.944	0.004**	0.630
delta pVO$_2$ (ml/FFM kg/min	0.645	0.073 to 0.897	0.032*	0.416
delta LVEF (%)	-0.339	-0.780 to 0.327	0.308	0.115
delta LV mass (g)	0.431	-0.228 to 0.819	0.186	0.186
delta BNP (pg/ml)	-0.159	-0.693 to 0.487	0.641	0.025
delta HF (ms^2)	0.070	-0.554 to 0.643	0.839	0.005
delta LF (ms^2)	0.454	-0.201 to 0.828	0.161	0.206
delta plasma NE (ng/l)	-0.029	-0.618 to 0.581	0.932	0.001
delta insulin (pmol/l)	0.478	-0.171 to 0.838	0.137	0.229
delta CRP (ng/l)	-0.019	-0.612 to 0.588	0.955	0.0003
delta Choesterol (mmol/l)	0.363	-0.340 to 0.775	0.272	0.132
delta glucose (mmol/l)	-0.192	-0.710 to 0.461	0.573	0.037

Table demonstrates correlations between FM changes from baseline to 16 weeks and other collected variables in patients with obesity and heart failure/ high CVD risk. (N=11) Correlation and significance were determined using Pearson's Correlation test. Correlation as significant* at the 0.05 level. FM=fat mass, FFM= fat free mass, pVO$_2$ = peak VO$_2$, LVEF=left ventricular ejection fraction, LV mass= left ventricular mass, CRP = BNP= b-type natriuretic peptide, HF=high frequency, LF= low frequency, NE=norepinephrine, c- reactive protein

Table 4. 12.   Correlation of changes in FFM (week 16 – 0) with other putative variables

Parameter	Pearson r	95% confidence interval	P value (two-tailed)	R squared
delta weight (kg)	0.637	0.060 to 0.895	0.035*	0.406
delta FM (kg)	0.281	-0.38 to 0.754	0.404	0.079
delta pVO2 (ml/kg/min)	-0.688	-0.912 to -0.150	0.019*	0.473
delta pVO2 (ml/min)	-0.161	-0.694 to 0.486	0.636	0.026
delta pVO2 (ml/FFMkg/min)	-0.391	-0.803 to 0.273	0.234	0.153
delta LVEF (%)	-0.167	-0.697 to 0.481	0.623	0.028
delta LV mass (g)	0.381	-0.284 to 0.799	0.248	0.145
delta BNP (pg/ml)	0.090	-0.539 to 0.655	0.792	0.008
delta HF (ms2)	-0.111	-0.667 to 0.524	0.745	0.012
delta LF (ms2)	0.184	-0.468 to 0.706	0.588	0.034
delta plasma NE (ng/l)	0.255	-0.407 to 0.7413	0.450	0.065
delta insulin (pmol/l)	0.304	-0.362 to 0.7647	0.363	0.093
delta CRP (ng/l)	0.106	-0.528 to 0.6635	0.757	0.011
delta cholesterol (mmol/l)	-0.320	-0.776 to 0.337	0.323	0.109
delta glucose (mmol/l)	-0.255	0.741 to 0.408	0.450	0.0648

Table demonstrates correlations between FFM changes from baseline to 16 weeks and other collected variables in patients with obesity and heart failure/ high CVD risk. (N=11) Correlation and significance were determined using Pearson's Correlation test. Correlation was significant* at the 0.05 level. FM=fat mass, FFM= fat free mass, pVO_2 = peak VO_2, LVEF=left ventricular ejection fraction, LV mass= left ventricular mass, CRP = BNP= b-type natriuretic peptide, HF=high frequency, LF= low frequency, NE=norepinephrine, c- reactive protein.

Figure 4. 16. **Correlation of changes in FM (week 16 -0) with changes in body weight and peak VO$_2$ (week 16 -0)**
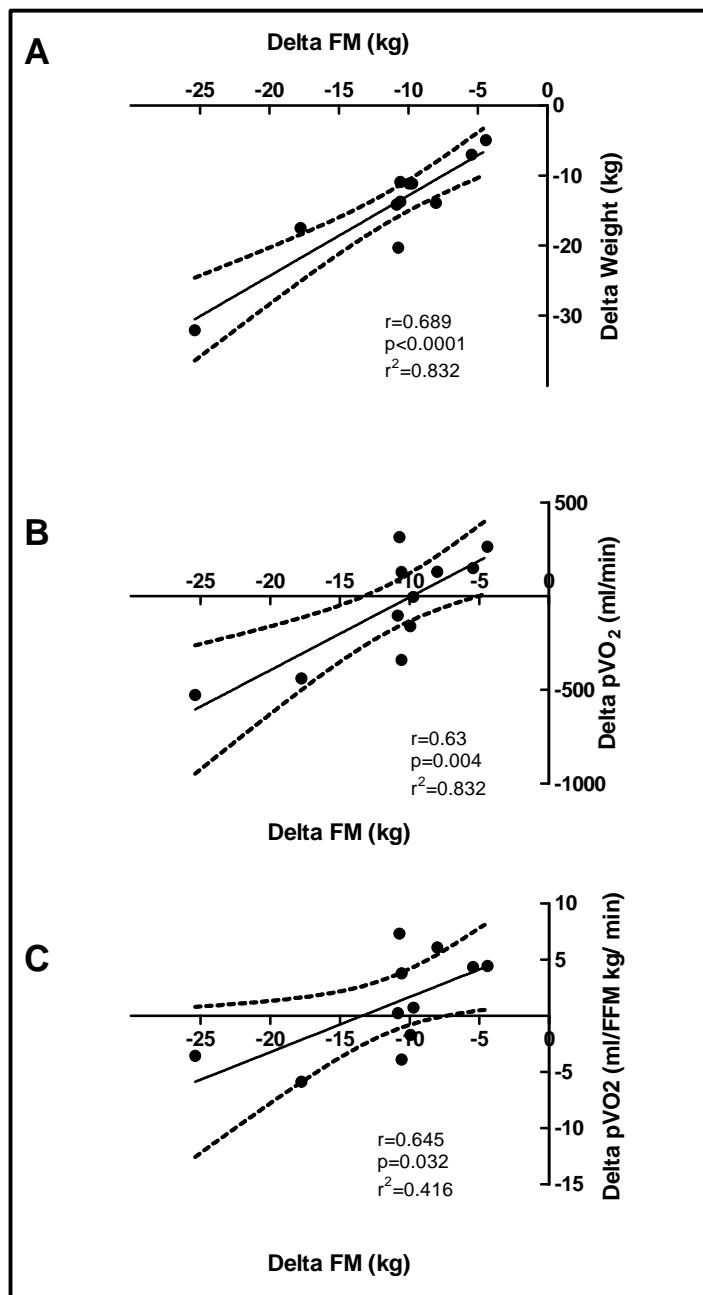
Delta FM correlated with delta body mass and delta pVO$_2$ (week 16-0) in obese patients with heart failure and or high cardiovascular risk. **A** represents correlation with body mass loss, **B** represents correlation with delta PVO$_2$ presented in ml/min (absolute value). **C** represents correlation with delta pVO$_2$ presented in ml/FFM kg/min. All graphs demonstrated significantly lower fat mass as delta pVO$_2$ decreased.

Figure 4. 17. Correlation of changes in FFM (week 16 -0) with changes in body weight and peak VO$_2$ (week 16 -0)
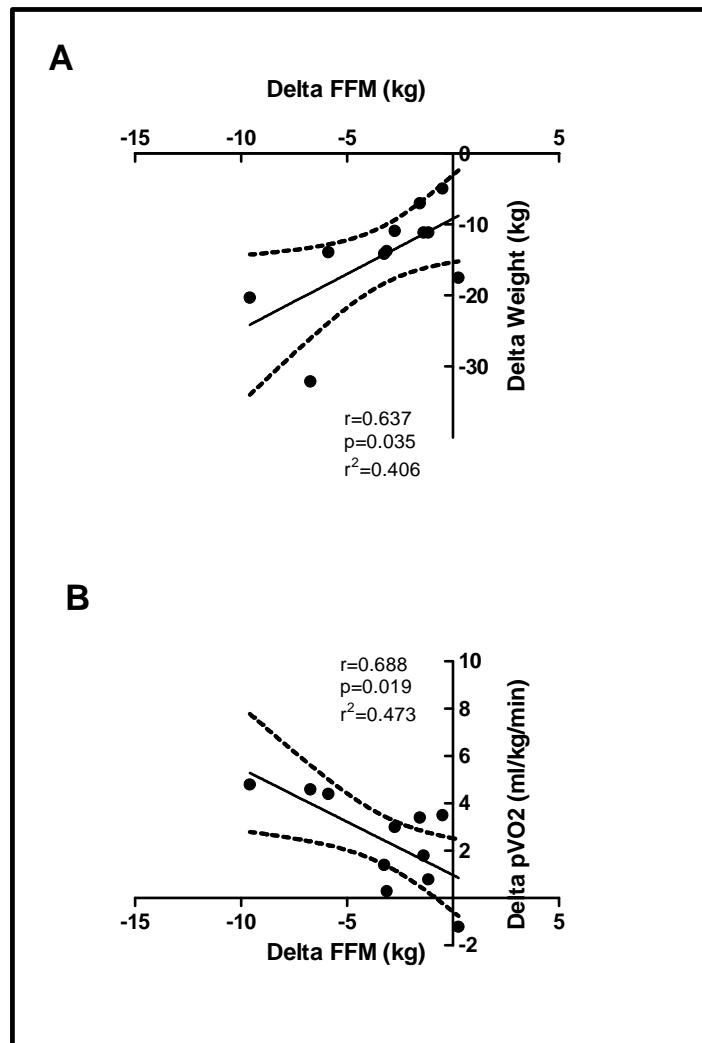
Delta FFM correlated with delta body mass and delta pVO$_2$ (week 16-0) in obese patients with heart failure and or high cardiovascular risk. **A** represents correlation with body mass loss, **B** represents correlation with delta PVO$_2$ presented in ml/kg/min (corrected by body mass). Graph **B** demonstrated significantly lower FFM as body mass decreased. Graph **B** demonstrated significantly increased in delta pVO$_2$ as FFM decreased.

4.12. Exploratory analysis of changes in fat mass and fat free mass - discussion

Both loss of FM and FFM occurred following the weight loss intervention. However, the degree of the correlation of weight loss was much stronger with FM loss than with FFM loss. In addition, there was no correlation in between loss of the FM and FFM i.e., FFM loss was independent and less of FM loss. This implied an achievement of a much healthier body composition following the therapeutic weight loss intervention.

Surprisingly, paradoxical correlations were noted between pVO_2 changes with the FM and FFM changes. Peak VO_2 changes correlated positively with delta FM (decreasing delta FM with decreasing delta pVO_2) and negatively with delta FFM (decreasing delta FFM with increasing delta pVO_2). The correlations were, however, not to a significance levels if pVO_2 unit was corrected with body mass (for correlation with FM) and as an absolute value and corrected by FFM (for correlation with FFM). Peak VO_2 measured during the cardiopulmonary exercise was influenced by various physiological statuses as stated in section 3.5.1and Figure 3.4**.** Factors that can influence O_2 availability are the oxygen carrying capacity of the blood (available haemoglobin, arterial O_2, CO_2 and dissociation curve shift with temperature, CO_2 and pH), cardiac function (heart rate, stroke volume, redistribution of peripheral blood flow, and by the tissues (capillary density, mitochondrial density and function, adequacy of perfusion, and tissue diffusion).[116] The skeletal muscle is a very important organ taking up O_2 from the circulation at maximal exercise (pVO_2). Paradoxically, the current study showed decreasing in lean body mass (FFM) correlated with increasing in pVO_2 following weight loss. In addition, adipose tissue does not have an oxygen carrying capacity and one would expect to see a reduction in fat mass correlating with increasing in pVO_2. Again, a paradoxical effect was seen in this study.

It was possible that those findings were be secondary to changes the composition of cardiac muscle following weight loss. In the current study, LV mass or LVEF measured by Echocardiography did not show any significant changes. However,

Echocardiography was technically difficult in obese individuals. In addition, Echocardiography was likely not sensitive to detect smaller changes occurred at short duration of the study. Evidence suggested that weight loss was associated with reduction epicardial fat (triglyceride) level and reduction in LV mass and improvement in diastolic function. Therefore, a detail cardiac composition measurement by cardiac MRI would be desirable.

CHAPTER 5:

LIMITATIONS, SUMMARY AND CONCLUSION

CHAPTER 5:   LIMITATIONS, SUMMARY AND CONCLUSION

5.1.   Limitations

5.1.1. Recruitment difficulty

This study was intended as an initial pilot study to be followed by a randomised controlled trial. Patients were recruited from cardiology, obesity and diabetes clinics. Recruitment was much harder than anticipated. With advances in medical therapy, stable heart failure is now mainly managed in the community. It was found that the majority of hospital clinic attendees were not suitable to take part in the study especially with the initial stringent inclusion criteria. Therefore, recruitment was extended by approaching cardiologists at Bedford Hospital, Luton and Dunstable hospital and West Suffolk Hospital as well as Cambridgeshire general practitioners (GP) including a GP with a special interest in cardiology and the community heart failure nurse. However, very little reposes were received. In retrospect, the study recruitment would have been improved in collaboration with other primary and secondary centres before the study was conducted. When hospital discharge letters were also searched for codes of the obesity (E66) and heart failure (I50), only 24 patients were identified. It was very likely that obesity was under recorded, and indeed the majority of these identified cases were in the extreme BMI of >40 kg/m^2. Interestingly, 5 of those patients identified died by the time of data search. The rest of the case notes were reviewed and potentially appropriate patients were approached by sending out invitation letters with prepaid envelops to reply back if there was any interest in participating in the study. Despite all these effort, only 3 patients were recruited at 9 months after the approval from the study by the Research ethic committee (REC). Therefore, a further amendment to the protocol was carried out with more relaxed criteria to include obese patients with high cardiovascular risks without the diagnosis of heart failure. The amendment was approved by REC (April 2007). The study was then, decided to base it on extended pilot study with the 11 patients who were on the active weight loss management programme. There was only one control patient who did not have the weight loss intervention and her data was not included in the analysis. This thesis was therefore based on the extended pilot study

without the control group and was thus under powered. Hence, the impact of natural history of disease on the changes seen could not be assessed.

5.1.2. Statistical analysis

At post hoc analysis, our study showed a greater improvement in peak VO_2 (13.2%) than predicted (10%) with a mean difference of 2.43 ml/kg/min (18.33 to 20.76 ml/kg/min). The standard deviation (SD) of the study was larger at 4.65 than estimate of 2.9. Having small sample size, the study was significantly under power at 34% (Table 5.1). Although it was under powered, the study has demonstrated the statistically and clinically significant improvement of the primary end point, peak VO_2. The study did not power for assessments of other (secondary) parameters. Since the study was significantly under power for the primary end point, confidence interval (CI) for those other parameters were large and "non significant" difference (NS) are subject to type 2 errors.

Table 5. 1.   Post hoc analysis: Power calculation

t tests - Means: Difference between two dependent means (matched pairs)			
Analysis:	Post hoc: Compute achieved power		
Input:	Tail(s)	=	Two
	Effect size dz	=	0.5199256
	α err prob	=	0.05
	Total sample size	=	11
Output:	Noncentrality parameter δ	=	1.7243981
	Critical t	=	2.2281389
	Df	=	10
	Power (1-β err prob)	=	0.3448195

Data were analysed with parametric test as planned. However because of the smaller sample size, non parametric tests would have been more appropriate. On analysis of the

primary end point data or log transformed data with histogram confirmed that the data were not normally distributed. Nevertheless, the analysis using non parametric Friedman test of primary end point (peak VO2) also showed a significant difference in the means with a "p value" of 0.0377. Post hoc analysis with Dunn multiple comparison test showed significant mean difference from week 0 to 16, but not significant from week 0 to 6. (Appendix. D)

Base on the study finding, it was calculated that 29 subjects would have been needed to detect a 13.2% improvement in peak VO_2 after a 13.5% weight loss at an effect size of 0.586, p value <0.05 and 85% power (Table 5.2). To achieve 3:1 intervention Vs non-intervention ratio, the number in intervention group was increased to 39 with an additional 10 patients were to be recruited as the non-intervention control group. If 10% drop out is anticipated, the study would require 43 patients. Different sample sizes could be examined that was plotted against power (Figure 5.1).

Table 5. 2.   Sample size calculation by G Power software (85% power)

t tests - Means: Difference between two dependent means (matched pairs)		
Analysis:	A priori: Compute required sample size	
Input:	Tail(s)	= Two
	Effect size dz	= 0.5863654
	α err prob	= 0.05
	Power (1-β err prob)	= 0.85
Output:	Noncentrality parameter δ	= 3.1576743
	Critical t	= 2.0484071
	Df	= 28
	Total sample size	= 29
	Actual power	= 0.8616441

Figure 5. 1   X-Y plot for a range of sample size against power
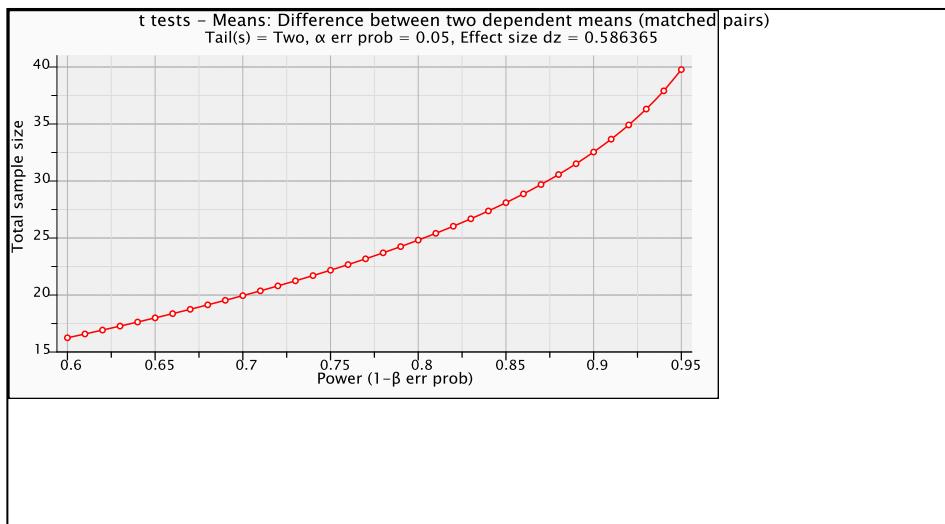
5.1.3. Study population

Our study was initially intended to conduct on obese patients with heart failure. It was then extended to include patients with high cardiovascular risk. As a result, there were only 4 patients with the diagnosis of heart failure who had completed the study. Being a study of a small sample size and having a heterogeneous group of cohort, some of the statistically insignificant results the study were prone for the type II errors. On the other hand, the current study cohort reflected a group of high cardiovascular risks and had provided a good reflection of intended study objectives.

5.2.   Summary

This study aimed to see whether WL or WLM was of benefit or harm to patients with heart failure and or high cardiovascular risk. While the "gold standard" for such a study would involve cardiac or other outcome events, an extremely large and long term study would be needed. Therefore, alternative parameters of cardiac performances (pVO_2), haemodynamic changes, sympathetic activity and metabolic profile were measured following active weight loss intervention. Detailed body composition changes

(measured by the techniques with high precision using four compartment model, whole body quantitative measurement of body fat by MRI) were performed. Weight loss intervention was carried out by providing low energy liquid diet of 3.35 MJ-4.19 MJ (800- 1000 Kcal/day) and weight loss maintenance was achieved by providing solid food which met 80% of daily energy requirement. Unfortunately, due to the recruitment difficulties, the study was largely limited by not having a control group. Thus, this data presented with in this thesis was based upon 11 pilot patients and under powered. In this cohort, the aim of the study was met achieving an acute weight loss and maintaining such weight loss toward the end of the study. Both the use of the specific diet and inducing rapid weight loss were proven to be safe in this study group. There were significant improvements in haemodynamic parameters, cardiac performance in terms of peak VO_2, sympathetic system activity and various metabolic profiles.

The question remains as to why there were consistent epidemiological data showing that low body weight in a group of patients with heart failure have a high risk of cardiovascular mortality.[27, 41, 152] More recently "the candesartan in heart failure: assessment of reduction in mortality and morbidity (CHARM) programme"[297] studied mortality of weight changes over 6 months in 6993 heart failure patients. The percentage weight loss over 6 months had an increasing association with excess mortality. Patients with 5% or greater weight loss in 6 months had over a 50% increased in hazard compared with those with stable weight.

However, in these studies, the fall in weight was not voluntary or as a result of a therapeutic weight loss. It was likely to reflect an advance in the cardiac disease, or could reflect unsafe weight loss practices (e.g. Atkin diets). It is possible that the short term nature of our study failed to see the deleterious effects but it seems unlikely. Therefore, long term larger outcome studies of weight loss in high risk cardiovascular patients are needed to answer the question. Jhaveri et al[298] showed a substantial weight loss (mean 32kg) following bariatric surgery was associated by significant reduction in LV mass (34%) and 16% RV mass measured by cardiovascular magnetic resonance

imaging. The Swedish Obese Subjects Study (SOS)[299, 300] followed 2000 patients who underwent bariatric surgery. With an average of 10 years of follow up, reduction of traditional cardiovascular risk factors and the overall risk factor-adjusted mortality (30.7%) were seen compared with the controlled group.

Though there were limitations, the present study also carried some major strength. The study was unique because it investigated detailed aspects of patient cardiovascular status including measurement of cardiac parameters (structure, function and performance), haemodynamic parameters and autonomic functions. This was carried out in parallel to measuring body compositions with a gold standard technique (4C) and comprehensive assessment of metabolic profile and cardiac risk biomarkers. In addition, the choice of the weight loss intervention with LCD was successful reflected by high retention rate and achievement of significant weight loss implying adherence to calorie restriction. The current study demonstrated the metabolic and clinical safety of weight loss in patients with heart failure and or high cardiovascular risk.

5.3.   Conclusion

In this pilot study (N=11), weight loss in patients with heart failure and or high CVD risk was associated with improvements in cardiac performance (pVO_2), haemodynamic parameters (reduction in systolic blood pressure, resting heart rates), autonomic function (reduction in resting heart rate, plasma and urine norepinephrine, and increase in HRV) and CVD risk profile (cholesterol, CRP) and metabolic profile (decrease in fasting glucose, insulin, ALT, TNF-α). The study will form a platform for future multicentre randomised control intervention outcome trials.

CHAPTER 6:

FURTHER DIRECTION

CHAPTER 6:   FURTHER DIRECTION

There was little evidence for the link between high body mass index and the better prognosis for heart failure patients and that there is need for well-controlled, large scale studies to explore this. Based on the current study, future work could be conducted for a larger multicentre control trial of weight loss intervention. In addition to investigating current study's end points, the future study should aim to investigate outcomes i.e., CV deaths and composite cardiovascular events (acute myocardial infarction, heart failure, peripheral vascular disease, End stage renal failure) and therefore will be a longer study (5 years). The cohort will be stratified into different sub-groups i.e., with or without heart failure, diabetes and metabolic syndrome. In term of body composition measurements, assessment of differential fat mass (e.g., visceral fat) with proton MR spectroscopy will be important. This should be compared with regional fat mass changes measured by DXA. More precise and advance cardiac measurement by Cardiac MRI should be included in the study to measure all cardiac parameters which would be available from echocardiogram as well as intramyocardial fat (triglyceride) level. To achieve a sustainable weight loss at longer period, WLM phase will be reinforced by a) use of different weight loss drugs including orlistat and newer glucagon like peptide-1 (GLP-1) agonists such as Exenetide and Liraglutide and b) life style modification (exercise program and calorie restriction). A sub-study could also look at the carotid intima-medial thickening and detailed of atherosclerosis assessment by Positron-emission tomography (PET) CT scanning. Other parameters of psychological issue associated with obesity can also be examined.

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APPENDICES

A.   Publications from current thesis

Paper

1) **Myint KS**, Napolitano A, Miller SR, Murgatroyd PR, Elkhawad M, Nunez DJ, Finer N.
Quantitative magnetic resonance (QMR) for longitudinal evaluation of body composition changes with two dietary regimens. *Obesity (Silver Spring)* Feb 2010;18(2):391-6.

Abstract

1) **Myint KS**, Northcott S, Heck P, Wright A, Murgatroyd P, Ashby M, Dutka D, Dhatariya K, Brown MJ, Finer N. Effects of rapid weight loss and short-term weight loss maintenance in obese patients with cardiovascular risks and/or heart failure. *the Obesity Society, Annual Scientific Conference*; Oct 2008

Quantitative Magnetic Resonance (QMR) for Longitudinal Evaluation of Body Composition Changes With Two Dietary Regimens

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We have recently reported a validation study of a prototype low-field strength quantitative magnetic resonance (QMR) instrument for measurement of human body composition (EchoMRI-AH). QMR was very precise, but underreported fat mass (FM) by 2–4 kg when compared to a 4-compartment (4C) model in this cross-sectional study. Here, we report the performance of an updated instrument in two longitudinal studies where FM was decreasing. Healthy obese volunteers were given a modest energy deficit diet for 8 weeks (study A) and obese patients with heart failure and/ or at high cardiovascular risk were prescribed a low energy liquid diet for 6 weeks (study B). FM was measured at the start and end of these periods by QMR, dual-energy X-ray absorptiometry (DXA) and 4C. A higher proportion of the weight lost came from fat in study A compared with study B, where loss of total body water (TBW) played a greater part. The intraclass correlation between QMR and 4C estimates of FM loss (ΔFat) was 0.95, but 20 of 22 estimates of ΔFat by QMR were lower than the corresponding estimate by the 4C model. Bland–Altman analysis demonstrated that estimates of FM loss by QMR were ~1.0 and 0.7 kg lower than those obtained with 4C ($P = 0.0008$) and DXA ($P = 0.049$), respectively. Measurement precision remained high. QMR measurement should prove valuable for quantifying modest changes of FM in small trials.

INTRODUCTION

Expanded body fat depots in obese individuals are associated with endocrine and metabolic disturbances that impact health and longevity. The usual measures of therapeutic outcome and benefit are based upon easily measurable surrogates such as body mass (BM) and BMI, but ultimately the treatment of obesity aims to reduce the excessive fat mass (FM). Unfortunately, at an individual level weight and BMI are indirect and imprecise estimates of body fat content, especially across ethnic groups (1), the young, the elderly (2) and those with illness or chronic disease (3). Under long-term moderate energy deficit, fat loss accounts for 75% of total weight loss, but during early or rapid weight loss, or in patients with disease states such as heart failure or diabetes (4), fat-free mass loss may be as much as 50% (5). Furthermore, individuals vary in their adiposity and lean BM, and BMI may misrepresent the extent of metabolic disturbance when, for example, an individual with a normal FM is categorized as overweight, and vice versa. Indeed weight loss induced by diet or pharmacotherapy may not accurately represent fat loss, and there is the

potential for misinterpreting the clinical benefit (or harm) from such interventions.

Accurate measurement of body composition is thus of critical importance in evaluating the efficacy, potential benefits, and risks of interventional weight loss. This is recognized by regulatory authorities in their guidelines relating to the development of antiobesity drugs. The US Food and Drug Administration (6) requires that studies "ensure that drug or biologic-induced weight loss is caused primarily by a reduction in fat content, not lean body mass," and the European Committee for Medicinal Products for Human Use similarly comment that "measurements using accepted methods selected and justified by the applicant should demonstrate that weight loss is associated with appropriate loss of body fat (as distinct from muscle or body water)" (7).

The currently accepted reference method for body composition brings together (i) BM, (ii) total body water (TBW) mass measured by stable isotope dilution (e.g., deuterium oxide, D_2O), (iii) body volume (BV) usually quantified by air displacement plethysmography, and (iv) bone mineral content

(BMC) obtained by dual-energy X-ray absorptiometry (DXA) in a 4-Compartment (4C) model from which absolute fat and lean masses are derived. A number of expressions have been derived to relate FM to these parameters; in our studies we have used the following expression (8):

$$FM = (2.747 \times BV) - (0.71 \times TBW) + (1.46 \times BMC) - (2.05 \times BM).$$

However, the measurement of the 4C components can be inconvenient and in practice FM measured by DXA is more widely adopted as a primary measure of body composition, due to its reliability and ready availability, but these measurements can be confounded by when body size exceeds the limits for the instrument.

Quantitative magnetic resonance (QMR) methodology has been widely adopted for measuring body composition in live, unanaesthetised small animals (9), and it has become the preferred technique for measuring longitudinal changes in their FM over time. QMR was further developed to provide a convenient, noninvasive measurement of human body fat that offered the potential to improve on existing methods in precision and ease of measurement. EchoMRI-AH (EchoMedical Systems, Houston TX) is a novel QMR instrument which uses tissue-specific nuclear magnetic resonance properties of protons to separate signals originating from fat, "lean" tissue and "free" water and "total body" water (10).

We have recently reported on the use of a prototype EchoMRI-AH QMR instrument to measure body composition (11). The instrument performed well in tests to simulate body composition change using bottles of canola oil, and the level of precision was impressively high. In detecting small changes of FM, it was three times more precise than other methods such as DXA and 4C models.

In our initial validation report (11), changes in body composition were simulated and we emphasized that the precision and accuracy of QMR-derived estimates for FM change would require confirmation in longitudinal studies where subjects gain or lose weight. Here we report such an evaluation. We have studied two separate groups of subjects undergoing dietetic manipulations to reduce energy intake and produce weight (fat) loss. The first consisted of healthy overweight or obese volunteers who were placed on a modest energy deficit diet and the second group were overweight and obese patients with heart failure and/or at high cardiovascular risk who lost weight rapidly following prescription of a low energy liquid diet. This article extends the evaluation of QMR methodology by comparing the FM changes measured by the new EchoMRI-AH instrument to those measured by the 4C and DXA methods in terms of their agreement and relative precision. We use this information to assess the impact of the precision estimates on the powering of studies designed to assess changes in FM by QMR.

METHODS AND PROCEDURES

The studies were conducted at Addenbrooke's Hospital, Cambridge, UK. The QMR instrument is located in the GlaxoSmithKline Clinical Unit in Cambridge, whereas the other measurements of body composition were performed in the adjacent Wellcome Trust Clinical Research Facility.

Human intervention studies

Study A took advantage of a randomized clinical trial of a weight loss agent (EUDRACT 2006-003864-71) to compare the performance of the QMR, 4C and DXA methods in the placebo-treated subjects. Thirty healthy subjects were recruited by direct advertisement via the GlaxoSmithKline Clinical Unit in Cambridge. Inclusion criteria included BMI between 30 and 40 kg/m^2 and age between 18 and 55 years. Subjects with type 2 diabetes mellitus were excluded. The use of recreational drugs, alcohol, caffeine, and strenuous exercise were forbidden. A qualified dietician advised subjects on a hypocaloric diet designed to produce a daily energy deficit of 2,510 kJ, based upon estimates of energy expenditure derived from standard equations (12) assuming a physical activity level of 1.3. After a 2 week run-in on diet alone, baseline measurements, including FM assessment, were made. Subjects were then randomized in a 3:2 ratio to receive an experimental weight loss compound ($n = 18$) or identical matched placebo tablets ($n = 12$). Subjects were counselled biweekly for 8 weeks, after that they again underwent measurement of body FM by QMR, 4C and DXA methods. Data presented here are from the 11 subjects who, after unblinding on completion of the study, were found to have been randomized to placebo. QMR measurements were performed between 07:00 and 08:00 in the fasted state. BV (BOD-POD), DXA and D$_2$O dilution were performed between 09:00 and 14:00 on the same day as QMR measurements. Body weight was measured in light clothing before QMR measurements.

In study B, 11 patients with heart failure or other cardiovascular risk factors were recruited from the obesity and cardiology clinics at Addenbrooke's hospital into a study designed to investigate the impact of rapid weight loss on cardiovascular safety and benefit (Cambridge Research Ethics Committee Reference 06/Q0108/37). The study included measurements of body composition, VO$_2$ max, cardiac function by echocardiography, autonomic nervous system activity and antioxidant status. QMR measurements were performed in the morning in a fasted state, whereas DXA, BV, and D$_2$O dilution were performed on the same day as QMR scans. Body weight was measured in light clothing before QMR measurements. Study inclusion criteria included BMI between 30 and 50 kg/m^2, stable heart failure patients of New York Heart Association class II or III or patients with additional cardiovascular risk and age between 25 and 70 years. Subjects were placed on a low energy liquid diet as routinely used to achieve initial weight loss by the obesity clinic service at Addenbrooke's Hospital. The diet was based on semiskimmed milk and provided 3,350–4,490 kJ/day (76–84 g protein), depending on the initial BMI. Patients were seen weekly during this phase. After 6 weeks the subjects were advised on a conventional diet designed to provide an energy intake that would maintain the weight for a further 10 weeks. Data on the 11 subjects who completed the initial 6 weeks and who had QMR and 4C FM measurements are included here.

The studies were performed in accordance with Good Clinical Practice guidelines and the 1996 version of the declaration of Helsinki. The experimental protocols were approved by the protocol review panel at GlaxoSmithKline, the Cambridge Local Research Ethics Committee and by the Addenbrooke's Hospital R&D office and the Wellcome Trust Clinical Research Facility Scientific Advisory Board. All patients gave written informed consent to participation.

Body composition measurements

QMR. The characteristics of the QMR have been described previously (11). Since that publication, the software algorithm for deconvoluting FM has been updated by the manufacturer. These alterations related primarily to estimates of TBW, but were also expected to improve the precision of the fat measurements.

At the start of each day the QMR instrument was calibrated according to manufacturer's instructions using 45 kg Canola rapeseed oil, which has comparable magnetic resonance properties to human fat.

The oil composition is: 7% saturated fat, 62% monounsaturated fat, 26% polyunsaturated fat, and 5% of other constituents. In both studies, the QMR measurements were made in triplicate over a 10 min period. Room temperature was maintained at 22.5 °C.

4C model

In this study, FM was estimated using the following 4C equation (8):

$$FM = [2.747 \times BV] - [0.71 \times TBW] + [1.46 \times BMC] - [2.05 \times BM]$$

where BV is in litres and all other variables are in kilograms.

BV was derived from a BOD POD (Life Measurement, Concord, CA) using its estimates of % body fat and BM as follows (13):

$$BV = \frac{(\%fat + 450) \times BM}{495}.$$

TBW was measured by D_2O dilution using a protocol designed in collaboration with the Medical Research Council Human Nutrition Research Group, Cambridge, UK, who also undertook the deuterium analysis (14). Subjects were fasted from 22:00 and at 06:00 the next morning they were woken and asked to void urine. A saliva sample and a sample of the voided urine were taken for D_2O analysis. A drink of D_2O-enriched water (100 g of 7% by mass D_2O in H_2O) was then administered, and further saliva samples were taken at 4, 5, and 6 h post dose. Up to the 6 h sample, the volume of all urine passed was recorded and aliquots were retained for D_2O analysis to correct for label lost from the body water pool.

DXA. Total body bone mineral mass (BMC), FM, and lean mass were estimated from DXA scans (GE Lunar Prodigy; software version 8.1 GE Lunar, Madison, WI). In study B, three subjects at their first assessment were too heavy for measurement on the DXA instrument. The initial visit DXA data from a further three subjects was excluded on the basis that, though within the weight range of the instrument, their bone mineral content and lean mass estimates showed implausible changes between this and subsequent visits, related, we believe, to the initial depth of tissue in the trunk. For the 4C model calculation in these subjects, bone mineral content for the first visit was assumed to be the same as the second visit. DXA data are presented for the remaining five subjects.

The "fixed-set" intraclass correlation coefficient (ICC_3) was calculated to assess the degree of methodological variation between QMR and 4C methods (the two methods are considered "fixed" by the study design) (15). However, a high ICC is necessary but not sufficient to compare two measurements, so in addition the Bland–Altman method(15) was used to compare the change in FM measurements obtained by QMR with those from the 4C and DXA methods. These statistical analyses were carried out using SAS Version 9 for Windows.

Sample-size calculations were performed to assess the impact of QMR precision on a range of hypothetical trial designs using variability estimates from our initial report and from the two studies presented in this article. Sample-size calculations were performed using PASS 2005.

RESULTS

Table 1 shows the baseline demographics and body composition of subjects, and their response to dietary restriction.

Subjects in study A were prescribed an average energy deficit of ~2.7 MJ/day over 8 weeks, and those in study B ~6.7 MJ/day over 6 weeks. Assuming a 30 MJ deficit should produce ~1 kg weight loss, we anticipated losses of 5 kg for study A (4.8 kg achieved) and 9.4 kg for study B (12 kg achieved). The measured losses of FM (by 4C) were 4.7 and 8.0 kg, respectively. These outcomes suggest good average compliance with the dietary interventions. Data also indicate that a greater proportion of

the weight loss in study A came from loss of fat than in study B, where loss of TBW (derived from the 4C methodology) played a greater part.

Some important similarities and differences between the studies are illustrated in **Figure 1a,b**. Loss of FM and BM was achieved by all subjects except for one in study A. The baseline BM and FM were considerably more heterogeneous, and the degree of weight loss much greater, in study B. These two interventions therefore provide a good range of fat loss with which to compare the measurement methods, as well as two different clinical samples with differing body composition with which to make comparisons.

The relationship between QMR estimates of FM loss (ΔFat) and those obtained with the 4C model is illustrated in a correlation plot (**Figure 2**). The intraclass correlation (ICC_3) is 0.95, which is high. However, a high ICC is not sufficient to demonstrate agreement because it is strongly influenced by the variance of the measurement in the population in which it is assessed. The lack of agreement can be seen by the fact that 20 of 22 estimates of ΔFat by QMR were lower than the corresponding estimate by the 4C model.

Bland and Altman plots are a more appropriate method to assess the degree of agreement (16). These plot the difference between two methods against the mean of the methods, disclosing the variability, any systematic difference between methods, and any tendency of the difference to be related to the mean, without assuming that either method has superior properties. **Figure 3a** demonstrates that measurements of FM loss by QMR are lower than those obtained with the 4C

Table 1 Baseline characteristics and body weight and composition changes

	Study	
	A (*n* = 11) (8 weeks)	B (*n* = 11) (6 weeks)
Sex (female:male)	4:7	3:8
Age (years) ± s.d.	42 ± 14	54 ± 10
BMI (kg/m²) ± s.d.	32 ± 1	38 ± 5
BM (kg) ± s.d.	96 ± 11	113 ± 23
Fat (kg) (QMR) ± s.d.	33 ± 6	49 ± 16
Fat (kg) (4C) ± s.d.	37 ± 5	51 ± 15
TBW (kg) ± s.d.	45 ± 8	48 ± 10
Loss of BM (kg) ± s.d.	4.8 ± 3.5	12.0 ± 4.6
Fat loss (kg) (QMR) ± s.d.	3.6 ± 3.0	7.1 ± 4.1
% Fat loss (QMR) ± s.d.[a]	74 ± 20[b]	56 ± 17
Fat loss (kg) (4C) ± s.d.	4.7 ± 3.3	8.0 ± 3.2
% Fat loss (4C) ± s.d.[a]	94 ± 26[b]	68 ± 12
TBW loss (kg) ± s.d.	0.3 ± 1.5	3.9 ± 2.6
% TBW Loss ± s.d.[a]	8 ± 23[b]	31 ± 13

BM, body mass; QMR, quantitative magnetic resonance; TBW, total body water; 4C, 4-compartment.

[a]Percentage losses of fat and TBW are calculated relative to loss of BM. [b]*n* = 8; three patients who lost <2 kg of total body weight are excluded from percentage calculations.
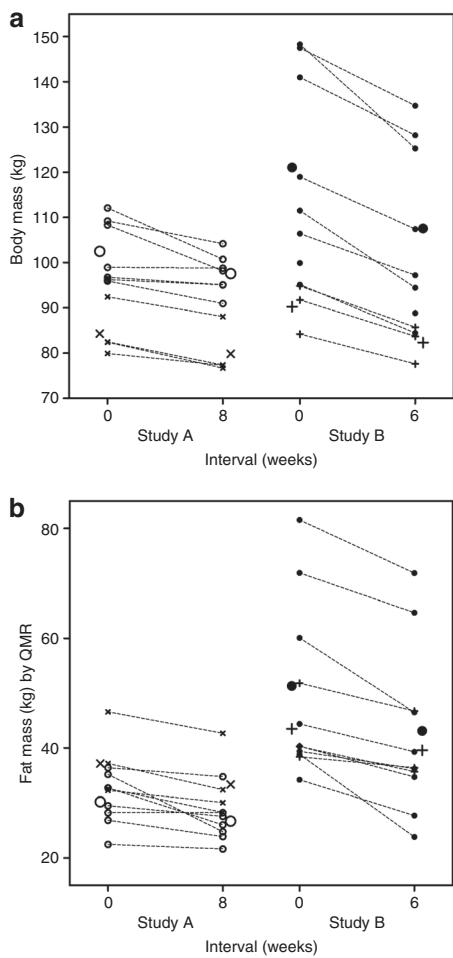
Figure 1 Mean values for female (×, +) and male (∘, •) subjects are shown with larger, offset symbols. Individual subject data are shown with smaller symbols joined by dotted lines.
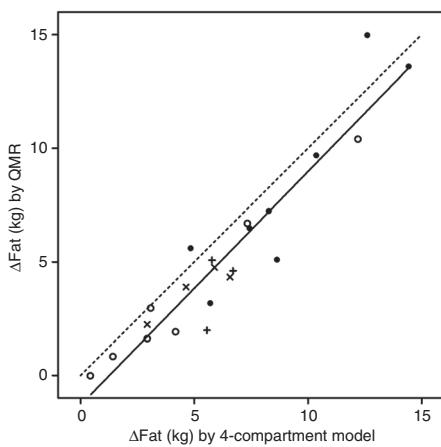
Figure 2 Study A female (×), male (∘) and study B female (+), male (•). The dotted line is the line of identity of the two methods. The solid line is a linear regression through the data points.

Figure 3 Study A female (×), male (∘) and study B female (+), male (•). The horizontal dotted line is the line of identity of the two methods. A linear regression through the data points is shown with a solid line, with dashed lines defining the boundaries of the 95% confidence interval.

model, similar to our previous data for absolute FM (11). The regression line shows no significant slope, but does demonstrate a mean difference of 1.03 kg ($P = 0.0008$). The standard deviation of the differences is 1.2 kg. Similarly, there was no significant slope when the difference between methods was plotted against absolute BM (data not shown). We note that these plots may generate an apparent systematic bias when the methods have different standard deviations (17). Assuming a standard deviation of 0.43 kg for QMR and 1 kg for 4C, we calculate that any bias would be <0.3 kg in the intercept, and ~0.06 in the slope. Therefore, this effect does not explain the difference between the methods, but may contribute to the observed nonsignificant slope.

Figure 3b shows the Bland and Altman plot comparing QMR to DXA fat loss. The conclusions are broadly similar, although the mean difference is slightly smaller (0.68 kg; $P = 0.049$). The spread of differences is comparable (standard

Table 2 Standard deviation of ΔFat between subjects (kg)

Standard deviation excluding measurement variance (σb)	0.5		0.75		1.0		2.0		3.0	
	QMR	4C	QMR	4C	QMR	4C	QMR	4C	QMR	4C
Total s.d. including measurement variance (σt)[a]	0.79	1.12	0.97	1.25	1.17	1.41	2.09	2.24	3.06	3.16
Difference in ΔFat between groups (kg)										
0.5	108	212	160	264	232	336	738	840	1,584	1,686
1.0	30	56	42	68	60	88	186	212	394	422
1.5	14	26	20	32	28	40	84	96	178	190
2.0	10	16	14	20	18	24	48	56	102	108
3.0	6	10	8	10	10	12	24	26	46	50
5.0	6	6	6	6	6	8	10	12	18	20

Sample-size calculations for fat loss studies using QMR/4C methodology. Sample sizes are total study size (two groups) assuming 90% power and 5% type 1 error. SD, standard deviation.

[a]Total s.d. including measurement variance (σ_t) is calculated as $\sigma_t^2 = \sigma_b^2 + \sigma_m^2$, where measurement s.d. (σ_m) of ΔFat by QMR is 0.61 kg, and by 4C is 1.0 kg.

deviation of differences 1.3 kg). Results are sparse for mean fat loss above 8 kg due to reliable DXA data only being available for five subjects.

For completeness we also include a Bland and Altman plot comparing DXA to 4C fat loss in **Figure 3c**. The 4C model estimates average loss of FM 0.19 kg greater than DXA ($P =$ 0.41). The standard deviation of the differences is 0.9 kg. There is some evidence that differences between methods is related to gender (mean difference DXA–4C is −0.86 kg for females and +0.21 kg for males; $P = 0.040$).

DISCUSSION

In our initial study, QMR provided a convenient method for measuring FM rapidly in humans (11). However, with the prototype that was employed in that study, there were some discrepancies in the absolute FM measurements when compared to the 4C model, probably due to the complex discrimination of fat and nonfat proton signals. On the other hand, simulated changes of FM (bottles of canola oil placed on the subject) were measured with a high level of accuracy and precision that was superior to the 4C model and DXA.

In this article, we have compared the performance of an updated QMR instrument, the 4C model and DXA to assess FM changes produced by two distinct interventions that aimed to decrease FM by reducing energy intake. These dissimilar cohorts and interventions were chosen to provide a broad spectrum of baseline phenotypes, as well as a wide range of alterations in FM with which to assess the precision of the QMR instrument and its agreement with the 4C model and DXA. The differences in the absolute changes in FM between study A and B were consistent with a daily energy deficit of ~2,500 kJ for study A and 6700 kJ for study B. When body composition changes were calculated as a percentage of the loss in BM, a greater proportion of this change was due to changes in TBW for the subjects in study B. This suggests that in the heart failure patients there was a more rapid depletion of glycogen reserves and perhaps a greater sodium and water diuresis than in healthy obese subjects, and therefore a greater decrease of TBW in the early phase of weight loss (18).

In terms of agreement, the QMR underreported the estimated fat loss (ΔFat) by an average of 1 kg when compared to the 4C model and DXA. This is somewhat better than our previous data on static assessments of FM (11), and may reflect the system upgrade between studies. No slope was detected when the difference between ΔFat by QMR and 4C was compared to the average of ΔFat by the two methods (**Figure 3**), indicating that the absolute difference between the methods is not affected by the magnitude of the fat loss. Similarly, the difference between methods was also not affected by absolute BM (data not shown). This is encouraging, and indicates that the QMR methodology can be used reliably regardless of the body composition of the overweight subjects at the time of initiation the intervention and the extent of the change in body composition during the intervention.

With regard to measurement precision of ΔFat for an individual subject, the variance of ΔFat will be simply twice the variance of the estimate of absolute FM at the start and end of the study (assuming independence between the two measurement "errors"). Based on our previous estimate of the standard deviation (the square-root of variance) of fat by QMR of 0.43 kg for subjects with BMI >30 kg/m^2, this gives an standard deviation for ΔFat for an individual subject of 0.61 kg (i.e., 0.43 kg × $\sqrt{2}$), which compares very favorably to other methodologies for body composition analysis.

The parameter of interest when designing a parallel-group study to compare two interventions is the variance of ΔFat between subjects. This variance can be split into two components: (i) the measurement variance as described above, and (ii) the variance between individuals in their response to intervention. This second "biological" variance is independent of the measurement methodology, instead being driven by aspects such as the homogeneity of the population, and the level of

compliance with the intervention. In study A and study B, the standard deviations of ΔFat subjects were between 3.0 kg and 4.1 kg, respectively, considerably larger than the pure "technical" measurement variation.

Table 2 shows sample-size calculations for a range of values of the standard deviation of ΔFat between subjects, and for the difference in ΔFat between groups. It is clear that for studies with larger standard deviation (columns to the right; standard deviation ≥2 kg) the reduction in measurement variance cannot sufficiently reduce sample size to make QMR-based studies feasible when compared with other technologies. In situations where the difference between interventions is larger (rows to the bottom; difference ≥2 kg), the sample size using the 4C method is generally small enough to make the studies feasible with this method anyway, and QMR would not provide an advantage other than cost and convenience. The studies where QMR may provide most benefit over 4C (or DXA) methods are those with smaller standard deviation (0.5–1 kg) and moderate difference (1–2 kg), where the sample size may be reduced by up to 40% (cells towards the top left). However, studies with very small differences (~0.5 kg) require large numbers of subjects whichever methodology is used (top row).

In conclusion, our data indicate that the EchoMRI-AH QMR methodology may be ideal for evaluating new antiobesity agents where preclinical promise has to be evaluated rapidly in humans to progress only those compounds that show significant efficacy. By focusing on FM as an inclusion criterion, successful interventions can be targeted to individuals most likely to respond because they have large fat depots at baseline. Our data suggest that this technology should be most advantageous for trials investigating moderate changes in FM, as in early phase trials, where sources of variation between individuals are kept to a minimum.

DISCLOSURE
The authors declared no conflict of interest.

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ABSTRACT

Effects of rapid weight loss and short-term weight loss maintenance in obese patients with cardiovascular risks and/or heart failure: a pilot study

Myint KS, Northcott S, Heck P, Wright A, Murgatroyd P, Ashby M, Dutka D, Dhatariya K, Brown MJ, Finer N.

Background: Despite the strong association of obesity with cardiovascular disease (CVD), several studies have shown that in patients with heart failure (HF) lower BMI increases mortality. Thus the benefits of weight loss in obese patients with established CVD/heart failure could be questioned.

Materials and methods: 11 obese patients with HF and/or additional CVD risks had measurements of body composition (4- compartment model), peak oxygen consumption (pVO_2), echocardiographic left ventricular ejection fraction (LVEF), and left ventricular mass (LVM) and other biomarkers: CRP, plasma norepinephrine (NE), leptin, adiponectin after 6 weeks of rapid weight loss (WL) (low energy liquid diet, 1200-1400 kcal/day) and then 12 weeks of weight loss maintenance (WLM). Data were analysed with analysis of variance and Bonferroni's multiple comparison post hoc analysis.

Results: All results mean $\pm$ SD. Baseline body weight 113.1kg $\pm$ 23.3 (range 84.2 to 148.3 kg). Total WL 10 and 12.2kg, fat WL 8.04 and 11.2 kg at week 6 and 18 respectively (ANOVA: $p < 0.0001$ for both). pVO_2 increased from 18.33 $\pm$ 4.5 to 20.96 $\pm$ 4.8 and 20.76 $\pm$ 4.8 ($p = 0.0095$). There were no significant LVEF or LV mass changes. NE (435, 328.4, 318.5ng/l; $p=0.03$) and CRP (4.452, 2.992, 3.25mg/l; $p=0.0088$) fell clinically and statistically significantly (weeks 0,6, 18). Leptin fell from 52.9 to 18.2 after rapid WL, but increased to 37.5µg/l at week 18 (ANOVA: $p=0.01$). Total cholesterol (4.2, 3.5, 3.9mmol/l; $p = 0.0005$) and triglyceride (1.9, 1.2; 1. 3mmol/l $p= 0.0037$) fell, with no significant changes in HDL and LDL cholesterol. ALT fell (41, 35.8; 26.8IU/l; $p=0.0368$). No overall change in glucose or blood pressure occurred, but some patients need to reduce anti-diabetic and anti-hypertensive medication.

Conclusions: Acute weight loss was associated with improvement in several CVD risk factors without any evidence of excess loss of lean body mass or reduction in cardiac performance. These findings are reassuring that both acute weight loss and maintenance in the short term of a lower body weight is not associated with evidence of increased CVD risk – in fact the reverse was observed. These data would support the safety of conducting larger, outcome studies of therapeutic weight loss in heart failure/CVD patients.

B. Information for Air displacement plethysmography (BOD-POD)

Wellcome Trust

Clinical Research Facility

**Measurement of
Body Composition by
BOD POD**

What is BOD POD?

The BOD POD is an instrument which measures body composition - the amount of fat and lean in your body.

How is it measured?

The BOD POD works out your body composition from your weight and the volume of your body. Your body volume is mesured from the amount of air space you take up when you sit inside the egg-shaped BOD POD capsule.

What does the measurement involve?

Before anything else, we will show you the BOD POD and explain the measurement procedure. Then we will measure your height. Next we will ask you to change into your bathing costume and put on a swimming hat that we will provide. This is to aviod any air being trapped between your clothing or hair and your skin. At the start of the measurement we will weigh you on the BOD POD's scale. Then we will ask you to sit in the BOD POD capsule for the body volume measurement. We will close and open the door two or three times during the measurement. You will find that, when the door is closed, you have a clear view through the large window in front of you. You yourself can open the door at any time if you wish.

How long does a BOD POD measurement take?

The total time you are in the BOD POD capsule for a measurement will be about 5 minutes. During this time, the door will only be closed for about 1½ minutes at a time. Most studies require a single measurement during your visit, though some require two. Occasionally it is necessary to repeat a measurement if we believe it may be inaccurate.

Will I be able to see the results?

The BOD POD gives results as soon as the measurement is complete. In most studies we are able to give you a printed copy of your results straight away, though in some studies the results may be given to you later.

C. Information for Dual Energy X-ray Absorptiometry

Wellcome Trust
Clinical Research Facility

**Bone Density and
Body Fat Measurement
by DXA**

What is a DXA?

DXA is an instrument which can measure the density of your bone and the amount of your body which is lean or fat. DXA stands for Dual-Energy X-ray Absorptiometer. It uses very low intensity X-ray to scan part or all your body.

What does the test involve?

Firstly, we will take time to explain the procedure to you and will encourage you to ask questions about the scan or if you are not sure what we want you to do.

During our explanation we will ask if you have any metal in your body e.g. plates and screws, and we will ask if you have had any other recent X-rays. This information helps us to interpret your measurements.

We will then ask you to lie on the DXA scanner couch. We will help you into the correct position for the part of your body we want to scan.
When the scan begins, the scanner arm, which carries the "camera", will pass over the part of the body to be scanned. It does not touch you.
It is important that you lie still during the scan, but breathe normally.

The scan does not involve any special drinks, injections or dietary restrictions.

Is radiation involved?

Yes, DXA is an x-ray measurement. However, the exposure from a DEXA is very small. We continually receive X-ray from the atmosphere, and the DXA X-ray exposure is equivalent to between 30 minutes and a few hours of atmosphirc X-ray, depending on the areas we scan. For comparison, a single chest X-ray is equivalent to about 5 days' atmospheric radiation.

What if I think I am pregnant?

We will not be able to scan you if you are pregnant. If you are female aged 12 to 50 years, we will ask if you could be pregnant. If there is any doubt we will ask your permission to perform a pregnancy test using a urine sample.

How will I be prepared for the scan?

We may take your height and weight measurements.

We will ask you to remove metal objects – e.g. watches, bracelets and spectacles. Rings and ear rings are not a problem.
You can wear your everyday clothing, but will need to remove your shoes. If possible avoid wearing any clothing with metal. If your clothing contains metal, such as zips or rivets, we may ask you to change into a gown or pyjamas which we will provide.

Preparing to scan the hip

Scanning the spine

How long does the scan take?

This will depend upon the area to be scanned, but 10 minutes at the most. Your visit should normally take not more than 30 – 45 minutes in total.

Will I be able to see the results?

After your scan the operator may be able to show you the images from you scan on the computer screen and may discuss the results with you.
You are welcome to have a printed copy of the image and results if the researcher is happy for you to have one.

Any other questions?

We hope this information is helpful. If you have any further queries please do not hesitate to ask.
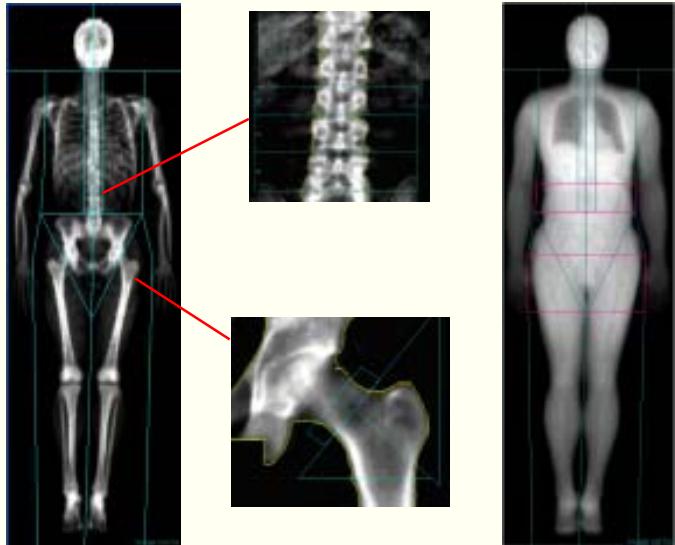
Spine, hip and whole body scan images

If you are particularly interested in the science behind your study, or in the measurements we make, then please ask - we will be delighted to spend some time discussing them with you.

Thank you for your interest in our research.

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D. Deuterium dilution – sample collection sheet

STUDY TITLE:	SUBJECT NUMBER:	DATE:
_____	_____	_ _ / _ _ / _ _

<u>Subject Log Sheet</u>

- **Anthropometric Data**

Weight of Subject	_ _· _ _ kg
Height of Subject (if collected)	_ _· _ _ m
Date of Dosing	_ _ / _ _ / _ _

- **Pre-dose saliva and urine**

Saliva collected	Yes/No
Urine collected(2ml)	Yes/No
Date	_ _ /_ _ /_ _
Time	............am/pm?

- **Deuterium dosing**

Dose Bottle Number	_____
Weight of **full** dose bottle + dose + straw	_ _ _ · _ _ g
Time dose given	.... am/pm?
Weight of **empty** dose bottle + straw	_ _ _ · _ _ g
Actual weight of dose (**full – empty**)	_ _ · _ _ g

- **4 hour saliva**   Yes/No

  Time   (4hour)   ....am/pm?

- **5 hour saliva**   Yes/No

  Time   (5hour)   ....am/pm?

- **6hour saliva**   Yes/No

  Time   (6hour)   ....am/pm?

BODY COMPOSITION ANALYSIS

PLEASE FOLLOW THE INSTRUCTIONS CAREFULLY

Saliva Sample Collection - small pieces of cotton wool are used to collect samples of saliva. To collect: -

(i)   Place cotton wool in the mouth and moisten for 60 seconds or until the cotton wool feels saturated.

(ii)   Whilst chewing, remove a 20ml syringe from the packet and take out the plunger from the barrel.

(iii)   Place the wet cotton wool into the barrel of the syringe and replace the plunger.

(iv)   Hold the syringe over a collection tube and depress the plunger to squeeze out the saliva into the tube.

(v)   Aim to fill each tube to the top with saliva at each collection time point, using extra pieces of cotton wool if necessary. A minimum of 1.0 ml is needed for analysis.

(vi)   Once enough saliva has been collected, the lid should be secured tightly onto each tube and the syringe thrown away.

Dosing

1)   On the day you start the study you must collect a <u>PRE DOSE SAMPLE</u>. It is VERY important that this sample is collected <u>BEFORE DRINKING</u> the special water (DOSE), and this sample should be put in the bottle marked 'Pre'. RECORD THE DATE AND TIME OF PRE COLLECTION ON YOUR FORM AND ON THE BOTTLE.

2)   DOSE: The dose has been labelled with a number. Record this number on the Log sheet in the appropriate space.

3)   The full dose bottle + cap and straw has to be weighed before drinking. Record the weight to two decimal places on the front of the sheet.

4)   Use the straw to carefully drink ALL the dose avoiding spillage and then crush the straw inside the empty bottle, replace the cap and re-weigh to two decimal places. Record this weight and also the exact time at which the dose was drunk. The actual weight of dose drunk can then be determined by taking away the empty weight from that of the full weight.

Sample Collection after the dose

1) Sample collection after the dose is at 4, 5, and 6 hours. Samples should be placed into appropriately labelled bottles and the dates and times of each collection recorded both on paper and on the bottles themselves just in case of loss of documentation. Fresh equipment should be used for each sample collection. DRINKING SHOULD BE AVOIDED THROUGHOUT THE STUDY PERIOD. HOWEVER, IF ABSOLTELY NECESSARY, IT IS IMPORTANT THAT SUBJECTS DO NOT DRINK FOR A MINIMUM OF HALF AN HOUR BEFORE COLLECTION OF ANY SAMPLE, DOING SO WILL CONTAMINATE THE NEXT SALIVA COLLECTION.

2) Collected samples should be frozen if possible. One subject's entire collection should be contained in one bag, included the pre dose separately in it's own bag.

3) At the end of the study the samples will be analysed and body composition determined at HNR in Cambridge.

E. Changes in Peak VO$_2$ analysed by non parametric Friedman test

Parameter			
Table Analyzed	peak VO2		
Friedman test			
P value	0.0377		
Exact or approximate P value?	Exact		
P value summary	*		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	3		
Friedman statistic	6.727		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
week 0 vs week 6	-10	No	ns
week 0 vs week 16	-11	Yes	*

F.   Samples of data Analysis work sheets

Parameter					
Table Analyzed	body weight				
Repeated Measures ANOVA					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	38.04				
R square	0.7919				
Was the pairing significantly effective?					
R square	0.8964				
F	83.17				
P value	< 0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1285	2	642.6		
Individual (between rows)	14048	10	1405		
Residual (random)	337.8	20	16.89		
Total	15671	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	12	6.846	Yes	***	7.419 to 16.58
week 0 vs week 16	14.2	8.104	Yes	***	9.624 to 18.78
week 6 vs week 16	2.205	1.258	No	ns	-2.374 to 6.783

Parameter					
Table Analyzed	BMI				
Repeated Measures ANOVA					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	44.75				
R square	0.8174				
Was the pairing significantly effective?					
R square	0.7978				
F	43.19				
P value	< 0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	145.7	2	72.86		
Individual (between rows)	703.2	10	70.32		
Residual (random)	32.56	20	1.628		
Total	881.5	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	4.005	7.361	Yes	***	2.584 to 5.427
week 0 vs week 16	4.802	8.827	Yes	***	3.381 to 6.224
week 6 vs week 16	0.7972	1.465	No	ns	-0.6242 to 2.219

Parameter					
Table Analyzed	4C Lean mass				
Repeated Measures ANOVA					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	18.05				
R squared	0.6435				
Was the pairing significantly effective?					
R squared	0.9619				
F	141.7				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	105.4	2	52.68		
Individual (between rows)	4137	10	413.7		
Residual (random)	58.38	20	2.919		
Total	4301	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week0 vs week 6	4.167	5.72	Yes	***	2.264 to 6.070
week0 vs week 16	3.242	4.451	Yes	***	1.339 to 5.145
week 6 vs week 16	-0.925	1.27	No	ns	-2.828 to 0.9782

Parameter					
Table Analyzed	4C fat mass				
Repeated Measures ANOVA					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	33.14				
R squared	0.7682				
Was the pairing significantly effective?					
R squared	0.865				
F	55.26				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	735	2	367.5		
Individual (between rows)	6129	10	612.9		
Residual (random)	221.8	20	11.09		
Total	7086	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week0 vs week 6	8.036	5.659	Yes	***	4.326 to 11.75
week0 vs week 16	11.21	7.897	Yes	***	7.505 to 14.92
week 6 vs week 16	3.179	2.239	No	ns	-0.5312 to 6.889

Parameter					
Table Analyzed	BMC				
Repeated Measures ANOVA					
P value	0.3414				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	1.135				
R squared	0.1019				
Was the pairing significantly effective?					
R squared	0.9504				
F	42.7				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.07035	2	0.03517		
Individual (between rows)	13.23	10	1.323		
Residual (random)	0.6199	20	0.031		
Total	13.92	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	-0.1119	1.491	No	ns	-0.3080 to 0.08422
week 0 vs week 16	-0.07009	0.9336	No	ns	-0.2662 to 0.1260
week 6 vs week 16	0.04182	0.557	No	ns	-0.1543 to 0.2380

Table Analyzed	FFDM	Fat free dry mass			
Repeated Measures ANOVA					
P value	0.0003				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	12.73				
R squared	0.56				
Was the pairing significantly effective?					
R squared	0.9425				
F	74.56				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	7.86	2	3.93		
Individual (between rows)	230.2	10	23.02		
Residual (random)	6.176	20	0.3088		
Total	244.2	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	1.021	4.309	Yes	**	0.4019 to 1.640
week 0 vs week 16	1.049	4.428	Yes	***	0.4301 to 1.668
week 6 vs week 16	0.02818	0.1189	No	ns	-0.5908 to 0.6472

Parameter					
Table Analyzed	Android and Gynoid ratio				
Repeated Measures ANOVA					
P value	0.0136				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	5.929				
R squared	0.4586				
Was the pairing significantly effective?					
R squared	0.871				
F	24.94				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.03637	2	0.01819		
Individual (between rows)	0.5355	7	0.07649		
Residual (random)	0.04294	14	0.003067		
Total	0.6148	23			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	0.0485	1.752	No	ns	-0.02675 to 0.1238
week 0 vs week 16	0.09535	3.444	Yes	*	0.02010 to 0.1706

Parameter					
Table Analyzed	heart rate				
Repeated Measures ANOVA					
P value	0.007				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	6.431				
R squared	0.3914				
Was the pairing significantly effective?					
R squared	0.7009				
F	7.701				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	881.2	2	440.6		
Individual (between rows)	5276	10	527.6		
Residual (random)	1370	20	68.51		
Total	7528	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	11.18	3.168	Yes	*	1.961 to 20.40
week 0 vs week 16	10.73	3.039	Yes	*	1.507 to 19.95
week 6 vs week 16	-0.4545	0.1288	No	ns	-9.675 to 8.766

Parameter					
Table Analyzed	diastolic BP				
Repeated Measures ANOVA					
P value	0.1011				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	2.576				
R squared	0.2048				
Was the pairing significantly effective?					
R squared	0.5912				
F	3.638				
P value	0.0067				
P value summary	**				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	328.8	2	164.4		
Individual (between rows)	2322	10	232.2		
Residual (random)	1277	20	63.83		
Total	3927	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	7.273	2.135	No	ns	-1.627 to 16.17
week 0 vs week 16	1.364	0.4003	No	ns	-7.536 to 10.26
week 6 vs week 16	-5.909	1.735	No	ns	-14.81 to 2.991

Parameter					
Table Analyzed	LVd mass				
Repeated Measures ANOVA					
P value	0.1115				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	2.453				
R squared	0.197				
Was the pairing significantly effective?					
R squared	0.8534				
F	14.5				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	5076	2	2538		
Individual (between rows)	150000	10	15000		
Residual (random)	20690	20	1035		
Total	175800	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	27.68	2.018	No	ns	-8.149 to 63.52
week 0 vs week 16	24.67	1.799	No	ns	-11.16 to 60.51
week 6 vs week 16	-3.01	0.2195	No	ns	-38.84 to 32.82

Parameter					
Table Analyzed	Exercise time				
Repeated Measures ANOVA					
P value	0.0013				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	9.402				
R squared	0.4846				
Was the pairing significantly effective?					
R squared	0.789				
F	14.51				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	89.48	2	44.74		
Individual (between rows)	690.3	10	69.03		
Residual (random)	95.17	20	4.759		
Total	875	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	-2.497	2.685	Yes	*	-4.927 to -0.06716
week 0 vs week 16	-3.992	4.292	Yes	**	-6.422 to -1.562
week 6 vs week 16	-1.495	1.607	No	ns	-3.925 to 0.9356

Parameter					
Table Analyzed	HF				
Repeated Measures ANOVA					
P value	0.0334				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	4.13				
R squared	0.3145				
Was the pairing significantly effective?					
R squared	0.4747				
F	2.637				
P value	0.0382				
P value summary	*				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	185700	2	92830		
Individual (between rows)	533500	9	59270		
Residual (random)	404600	18	22480		
Total	1124000	29			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	-27.59	0.4115	No	ns	-204.6 to 149.4
week 0 vs week 16	-179	2.669	Yes	*	-355.9 to -2.000
week 6 vs week 16	-151.4	2.257	No	ns	-328.3 to 25.59

Parameter				
Table Analyzed	fasting glucsoe			
Repeated Measures ANOVA				
P value	0.1257			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	2.304			
R squared	0.1873			
Was the pairing significantly effective?				
R squared	0.8026			
F	10			
P value	P<0.0001			
P value summary	***			
Is there significant matching? (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	6.764	2	3.382	
Individual (between rows)	146.8	10	14.68	
Residual (random)	29.36	20	1.468	
Total	182.9	32		
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary
week 16 vs week 0	-1.091	2.986	No	ns
week 16 vs week 6	-0.3727	---	No	ns
week 6 vs week 0	-0.7182	---	No	ns

Parameter					
Table Analyzed	HbA1c				
Repeated Measures ANOVA					
P value	0.0117				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	5.753				
R squared	0.3899				
Was the pairing significantly effective?					
R squared	0.8014				
F	13.23				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	4.266	2	2.133		
Individual (between rows)	44.15	9	4.905		
Residual (random)	6.674	18	0.3708		
Total	55.09	29			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	0.63	2.313	No	ns	-0.08867 to 1.349
week 0 vs week 16	0.9	3.305	Yes	*	0.1813 to 1.619

Parameter					
Table Analyzed	Choleterol				
Repeated Measures ANOVA					
P value	0.0005				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	11.35				
R squared	0.5316				
Was the pairing significantly effective?					
R squared	0.6572				
F	8.187				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	2.839	2	1.419		
Individual (between rows)	10.24	10	1.024		
Residual (random)	2.501	20	0.1251		
Total	15.58	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	0.7091	4.702	Yes	***	0.3151 to 1.103
week 0 vs week 16	0.2545	1.688	No	ns	-0.1394 to 0.6485
week 6 vs week 16	-0.4545	3.014	Yes	*	-0.8485 to -0.06059

Parameter					
Table Analyzed	HDL				
Repeated Measures ANOVA					
P value	0.0526				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	3.425				
R squared	0.2551				
Was the pairing significantly effective?					
R squared	0.7411				
F	7.685				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.09804	2	0.04902		
Individual (between rows)	1.1	10	0.11		
Residual (random)	0.2863	20	0.01431		
Total	1.484	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	0.08818	1.729	No	ns	-0.04510 to 0.2215
week 0 vs week 16	-0.04273	0.8375	No	ns	-0.1760 to 0.09056
week 6 vs week 16	-0.1309	2.566	No	ns	-0.2642 to 0.002375

Parameter					
Table Analyzed	Creatinine				
Repeated Measures ANOVA					
P value	0.2885				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	1.323				
R squared	0.1169				
Was the pairing significantly effective?					
R squared	0.9101				
F	22.93				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	291.9	2	145.9		
Individual (between rows)	25290	10	2529		
Residual (random)	2205	20	110.3		
Total	27780	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	4	0.8933	No	ns	-7.698 to 15.70
week 0 vs week 16	7.273	1.624	No	ns	-4.426 to 18.97
week 6 vs week 16	3.273	0.7309	No	ns	-8.426 to 14.97

Parameter					
Table Analyzed	CRP				
Repeated Measures ANOVA					
P value	0.0088				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	6.228				
R squared	0.409				
Was the pairing significantly effective?					
R squared	0.8792				
F	24.64				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	12.14	2	6.072		
Individual (between rows)	216.2	9	24.02		
Residual (random)	17.55	18	0.9749		
Total	245.9	29			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	1.46	3.306	Yes	*	0.2946 to 2.625
week 0 vs week 16	1.202	2.722	Yes	*	0.03663 to 2.367
week 6 vs week 16	-0.258	0.5843	No	ns	-1.423 to 0.9074

Parameter					
Table Analyzed	adiponectin				
Repeated Measures ANOVA					
P value	0.4065				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	0.9468				
R squared	0.09519				
Was the pairing significantly effective?					
R squared	0.7396				
F	6.277				
P value	0.0005				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	3.469	2	1.734		
Individual (between rows)	103.5	9	11.5		
Residual (random)	32.97	18	1.832		
Total	139.9	29			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	0.77	1.272	No	ns	-0.8274 to 2.367
week 0 vs week 16	0.11	0.1817	No	ns	-1.487 to 1.707
week 6 vs week 16	-0.66	1.09	No	ns	-2.257 to 0.9374

Parameter					
Table Analyzed	ALT				
Repeated Measures ANOVA					
P value	0.0368				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	3.913				
R squared	0.2813				
Was the pairing significantly effective?					
R squared	0.5288				
F	3.123				
P value	0.0145				
P value summary	*				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1133	2	566.5		
Individual (between rows)	4520	10	452		
Residual (random)	2895	20	144.8		
Total	8548	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	5.182	1.01	No	ns	-8.221 to 18.58
week 0 vs week 16	14.18	2.764	Yes	*	0.7787 to 27.58
week 6 vs week 16	9	1.754	No	ns	-4.403 to 22.40

Parameter					
Table Analyzed	antioxident profile				
Repeated Measures ANOVA					
P value	0.0009				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	10.2				
R squared	0.505				
Was the pairing significantly effective?					
R squared	0.7428				
F	11.67				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	219100	2	109600		
Individual (between rows)	1253000	10	125300		
Residual (random)	214800	20	10740		
Total	1687000	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	126	2.851	Yes	*	10.52 to 241.4
week 0 vs week 16	197.1	4.46	Yes	***	81.62 to 312.5
week 6 vs week 16	71.11	1.609	No	ns	-44.34 to 186.5

Parameter					
Table Analyzed	BNP				
Repeated Measures ANOVA					
P value	0.2037				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	1.725				
R squared	0.1471				
Was the pairing significantly effective?					
R squared	0.949				
F	43.67				
P value	P<0.0001				
P value summary	***				
Is there significant matching? (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	2355	2	1178		
Individual (between rows)	298100	10	29810		
Residual (random)	13650	20	682.7		
Total	314100	32			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
week 0 vs week 6	18.56	1.666	No	ns	-10.54 to 47.67
week 0 vs week 16	1.364	0.1224	No	ns	-27.74 to 30.47
week 6 vs week 16	-17.2	1.544	No	ns	-46.31 to 11.91