



Lifestyle and cardio-metabolic health

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Abstract

Type 2 diabetes is the fastest growing health threat to the UK, with prevalence rising 60% over the past decade. Those with Type 2 diabetes carry twice the risk of developing cardiovascular disease, a condition which claims the lives of the majority of adults in the UK. A significant proportion of cardio-metabolic disease could be prevented through improvements in lifestyle. Technological advancements, motorised transport and an increase in desk based work, have paved the way for physical inactivity to be norm in modern society. Clinical and government strategies to target unhealthy lifestyles are currently lacking.

The aim of this thesis was to explore lifestyle related behaviours in cardio-metabolic disease, with a view to improving clinical care. A UK population based study (n=502,664) demonstrates that those with cardio-metabolic disease are characterised by low physical activity, sedentary behaviour and poor sleep. Combining all three behaviours exposes individuals to greater cardio-metabolic risk. A cross-sectional study (n=57) indicates that there are significant cardiac abnormalities in those with metabolic disease in the absence of overt heart disease. Finally, a randomised controlled trial (n=28) provides evidence that exercise can be used as a therapeutic strategy to improve cardiac structure and function in adults with Type 2 diabetes, and thereby moderate cardiac risk in this patient group.

This thesis delivers two clear messages; 1) lifestyle behaviours remain significant unaddressed risk factors and 2) physical activity and exercise strategies should be used as therapies to reduce risk and improve cardio-metabolic health. Looking ahead, the results from the this study highlight the need for lifestyle behaviours to be part of the prevention and management strategies for cardio-metabolic health, and support the NHS's 5 year plan to encourage healthier lifestyles as a priority.

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Author contributions

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The NAFLD and control data presented in chapter 4 was collected, analysed and published by Dr Kate Hallsworth (Hallsworth et al. 2012 *Journal of Hepatology*). The author collected and analysed the Type 2 diabetes data. Magnetic resonance imaging scanning was undertaken by the Newcastle Magnetic Resonance Centre radiographers. Data interpretation, statistical analysis, and publication of manuscript, was performed by the author with assistance from Dr Kieren Hollingsworth.

Patients were recruited and consent obtained by the author and Josh Wood. Data collection for methods outlined in chapters 3-5 (apart from magnetic resonance imaging) was performed by the author, with assistance from Dr Christian Thoma, Dr Kate Hallsworth and the nurses within the Clinical Research Facility. Data interpretation, statistical analysis and publication of manuscript for chapter 5 was performed by the author with supervisory help from Dr Djordje Jakovljevic and Professor Mike Trenell.

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Publications, conference proceedings, presentations and awards

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List of abbreviations

ACSM- American college of sports medicine

ADA- American diabetes association

AGE- Advanced glycation end products

ALP- Alkaline phosphatase

ALT- Alanine aminotransferase

AST- Aspartate aminotransferase

ATP- Adenosine triphosphate

AUC- Area under the curve

BMI- Body mass index

Ca²⁺-Calcium

CHO- Carbohydrate

CVD- Cardiovascular disease

DAG- Diacylglycerol

DNL- De novo lipogenesis

DPG- Diphosphoglycerate

E/A- Early to late diastolic filling ratio

ECG- Electrocardiogram

EPOC- Exercise post oxygen consumption

G6P- Glucose 6 phosphate

GGT- Gamma-glutamyltransferase

GLUT 2- Glucose transporter 2

GLUT 4- Glucose transporter 4

HbA_{1c}- Glycated haemoglobin

HDL- High density lipoprotein

HIIT- High Intensity Intermittent Training

HOMA2- Homeostasis model assessment 2

IFG- Impaired fasting glucose

IGT- Impaired glucose tolerance

IPAQ- International physical activity questionnaire

IR- Insulin resistance

METs- Metabolic equivalents

MRI- Magnetic resonance imaging

MRS- Magnetic resonance spectroscopy

NAFLD- Non-alcoholic fatty liver disease

NEFA- Non-esterified fatty acids

NICE- National Institute for Health and Care Excellence

OGTT- Oral glucose tolerance test

O₂- Oxygen

PARQ- Physical Activity Readiness Questionnaire

PCR/ATP- Phosphocreatine/Adenosine-triphosphate

PGC1- α -Peroxisome proliferator-activated receptor-gamma coactivator 1
alpha

PKC θ - Theta isoform of protein kinase C

PKC ϵ - Epsilon isoform of protein kinase C

PPAR α - Peroxisome proliferator-activated receptor α (PPAR α)

RCT- Randomised controlled trials

RER- Respiratory exchange ratio

RM- Repetition maximum

ROS- Reactive oxygen species

RPM- Revolutions per minute

TSR- Torsion to shortening ratio

TV- Television

VLCD- Very low calorie diet

VLDL- Very low density lipoprotein

VCO₂- Carbon dioxide elimination

VO₂- Oxygen consumption

VO_{2peak}– Peak oxygen consumption

Chapter 1 Introduction and literature review

1.2 General introduction

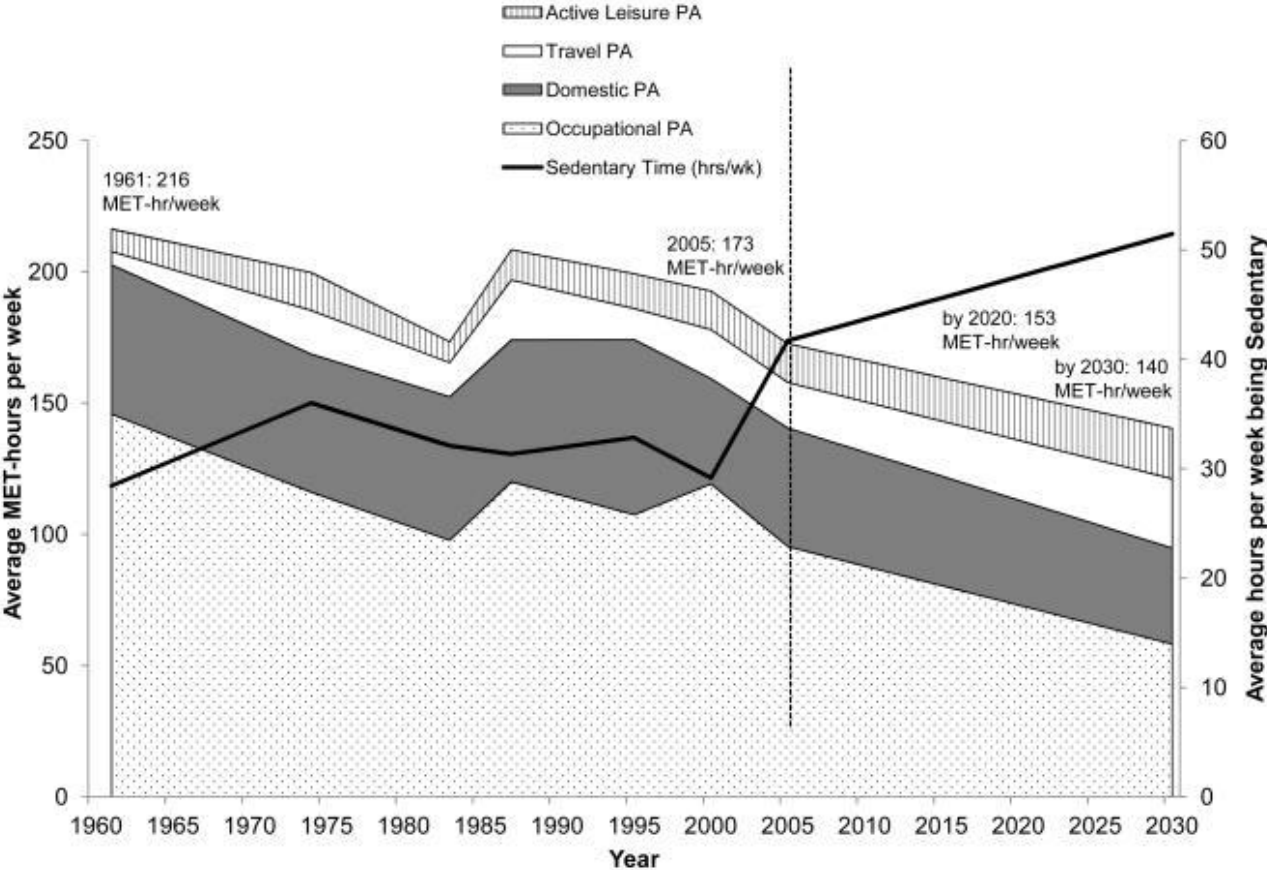
Diabetes is currently the fastest growing health threat to the UK, with >700 people being diagnosed every day in England (90% of diabetes cases are Type 2 diabetes) (Diabetes UK 2015). The economic impacts of this are substantive; current costs are £23.7 billion (10% of NHS costs) which is predicted to rise to 17% by 2035 (Hex et al. 2012). Global patterns show a similar trend, and predictions suggest that in two decades time prevalence will have risen by more than 75% (IDF 2013). Type 2 diabetes is clearly one of the biggest personal, economic and social challenges facing the 21st century and major action needs to be taken in the prevention, management and treatment of this chronic condition.

The majority of those with Type 2 diabetes die with heart disease (IDF 2013). The strong links between heart disease and Type 2 diabetes is often termed 'cardio-metabolic disease', and although their relationship is not fully understood, they share common environmental and genetic antecedents. Despite the large prevalence of cardiac dysfunction in Type 2 diabetes, treatment strategies are lacking.

Treatment and management strategies for cardio-metabolic disease support lifestyle changes before any pharmacological treatment (Inzucchi et al. 2012). Evidence shows that around 90% of Type 2 diabetes cases could be prevented through changes in lifestyle (Hu et al. 2001). Physical inactivity is the 4th leading cause of disease worldwide (Kohl et al. 2012) and technological advancements of modern society have paved the way for sedentary lifestyles to become the norm (Figure 1). If current trends continue we will be 35% less active by 2030 than in 1961 (Ng & Popkin 2012) which will have huge health consequences.

This literature review will describe normal metabolic control, lifestyle behaviours which influence metabolism, and cardiac structure and function, before moving onto to focus on these three areas in relation to Type 2 diabetes.

Figure 1 UK adults physical activity levels . Measured 1961-2005, forecast 2006-2030 (Ng & Popkin 2012). Measured using national surveys with MET intensities applied to activities within different domains.

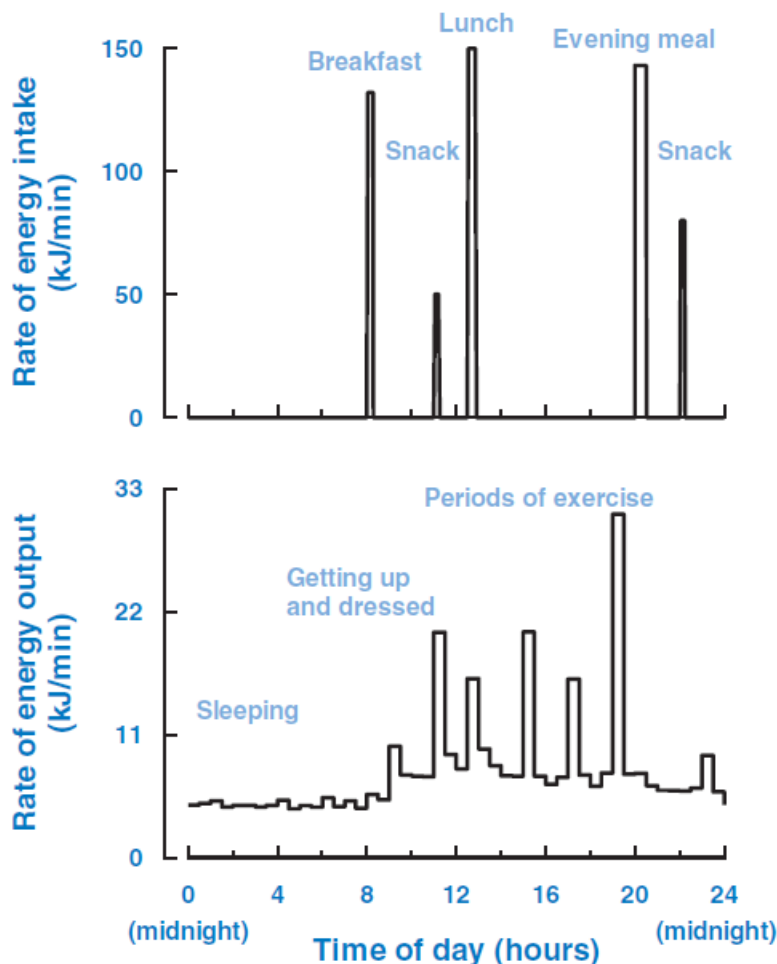


1.3 Metabolism

1.3.1 Overview

Metabolism can be defined as the life-sustaining chemical processes that occur in cells and organisms. A cardinal metabolic process is the conversion of chemical energy (in the form of food) into other forms of energy which can be used for all types of work from cellular to physical work. Metabolic control refers to the tight regulation of the body to store and release energy to maintain homeostasis despite the fluctuations in energy intake and output during the day (Frayn 2013) (Figure 2). This regulation enables us to lead normal lives in the face of a changing environment.

Figure 2 Fluctuations in energy intake and output for a person during a typical day (Frayn 2013)



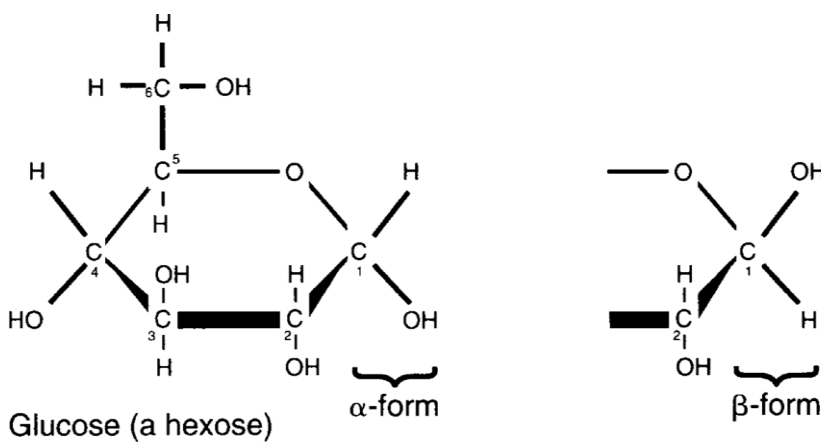
Such is the importance of metabolic control that even minor deviations can lead to metabolic diseases which impact upon health and wellbeing. The main energy sources in the body are derived from the macronutrients lipid,

carbohydrate (CHO) and protein but for the purpose of this review, only fat and carbohydrate will be considered.

1.3.2 Carbohydrate metabolism

Monosaccharides are basic CHO units and are classified according to the number of carbon atoms they hold. Common monosaccharides are hexoses (6-carbon atoms), fructose, galactose and glucose which is the most abundant monosaccharide in our diet and bodies and is the fuel which is used to supply energy for living organisms (Figure 3).

Figure 3 The structure of glucose in its ring α and β form (Frayn 2013).

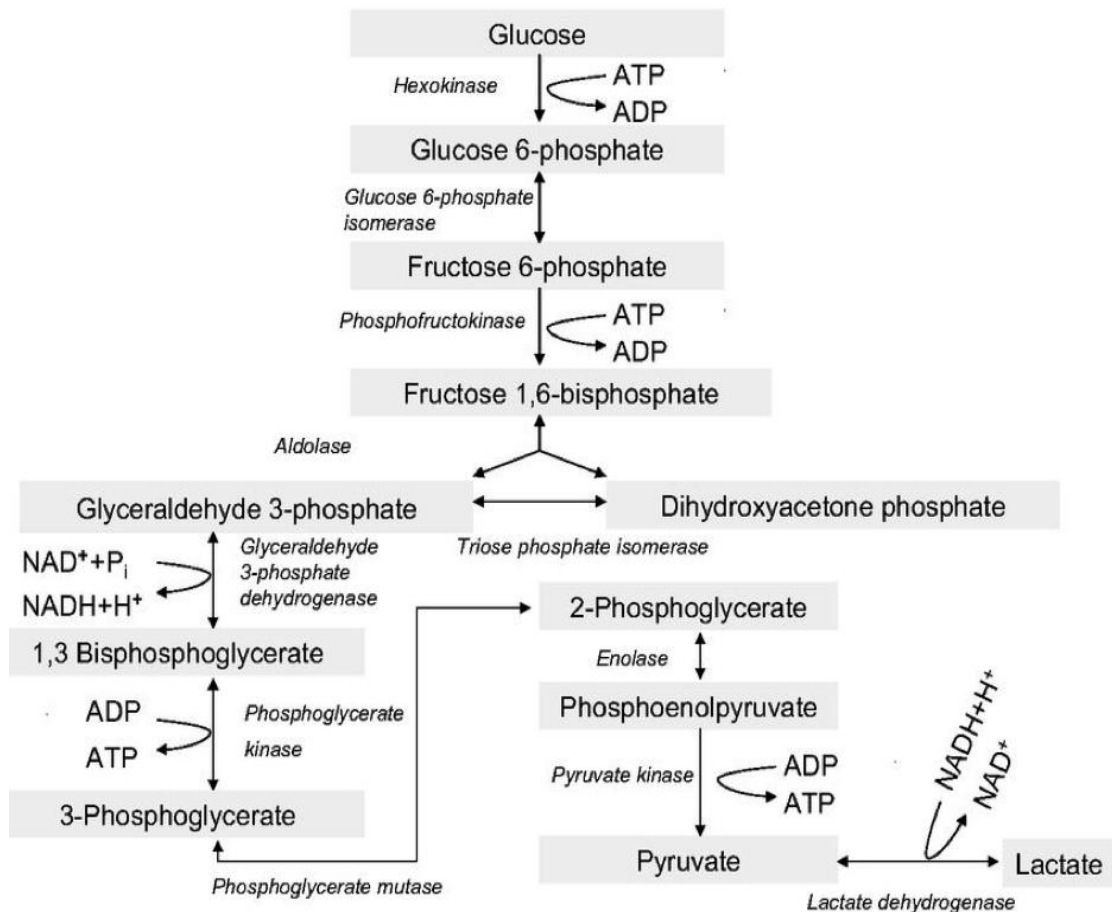


Glucose is soluble and circulates freely in blood but requires specific carrier proteins for entry into cells (Frayn 2013). Multiple glucose units are stored in cells as polysaccharide chains known as glycogen. Due to its water soluble nature, storage of glycogen is heavy as it carries around three times its own weight of water (Frayn 2013). Glucose can enter the bloodstream in 3 different ways; absorption from the small intestine, glycogenolysis and gluconeogenesis in the liver.

Energy is released from glucose via non-oxidative and oxidative pathways. Glycolysis (non-oxidative pathway) occurs in the cytoplasm and involves the partial oxidation of glucose to pyruvate (or lactate in the absence of oxygen) (Dashty 2013) (Figure 4). Within the complete degradation of one glucose molecule, glycolysis produces only around 5% of total adenosine triphosphate (ATP) however rapid reactions within this pathway make it crucial for bouts of maximal activity lasting up to 90 seconds. The first step in

glycolysis involves the phosphorylation of glucose to glucose 6-phosphate (G6P) by hexokinases which "traps" glucose within the cell (Figure 4). Subsequently, either *glycogen synthase* will act upon the molecule for glycogen storage or glucose breakdown will pursue; the fate of which is dependent upon the presence of hormonal regulators (Dashty 2013).

Figure 4 Schematic of glycolysis. Image taken from pg 579 (Ronquist et al. 2013).



The citric acid cycle and the electron transport chain (oxidative pathway) occur in the mitochondria in the presence of oxygen (Dashty 2013) (Figure 5). Pyruvate dehydrogenase converts pyruvate into acetyl-coA which enters the Krebs Cycle to release energy in the form of guanosine triphosphate and reduced forms of NADH and FADH₂. NADH is shuttled to the Electron Transport Chain; its hydrogen is released and as the electrons are transferred through a series of enzymes (complex I-V), energy is released (Figure 5). This energy from substrate level phosphorylation is used to create a proton gradient across the mitochondrial membrane, the reverse movement

of protons over the membrane releases energy and generates ATP (Dashty 2013) (Figure 5). Theoretically, oxidation of one glucose molecule produces 36 ATP molecules which is equivalent to 288 kcal however 56% of this energy is wasted as heat due to leaky mitochondrial membranes (Dashty 2013).

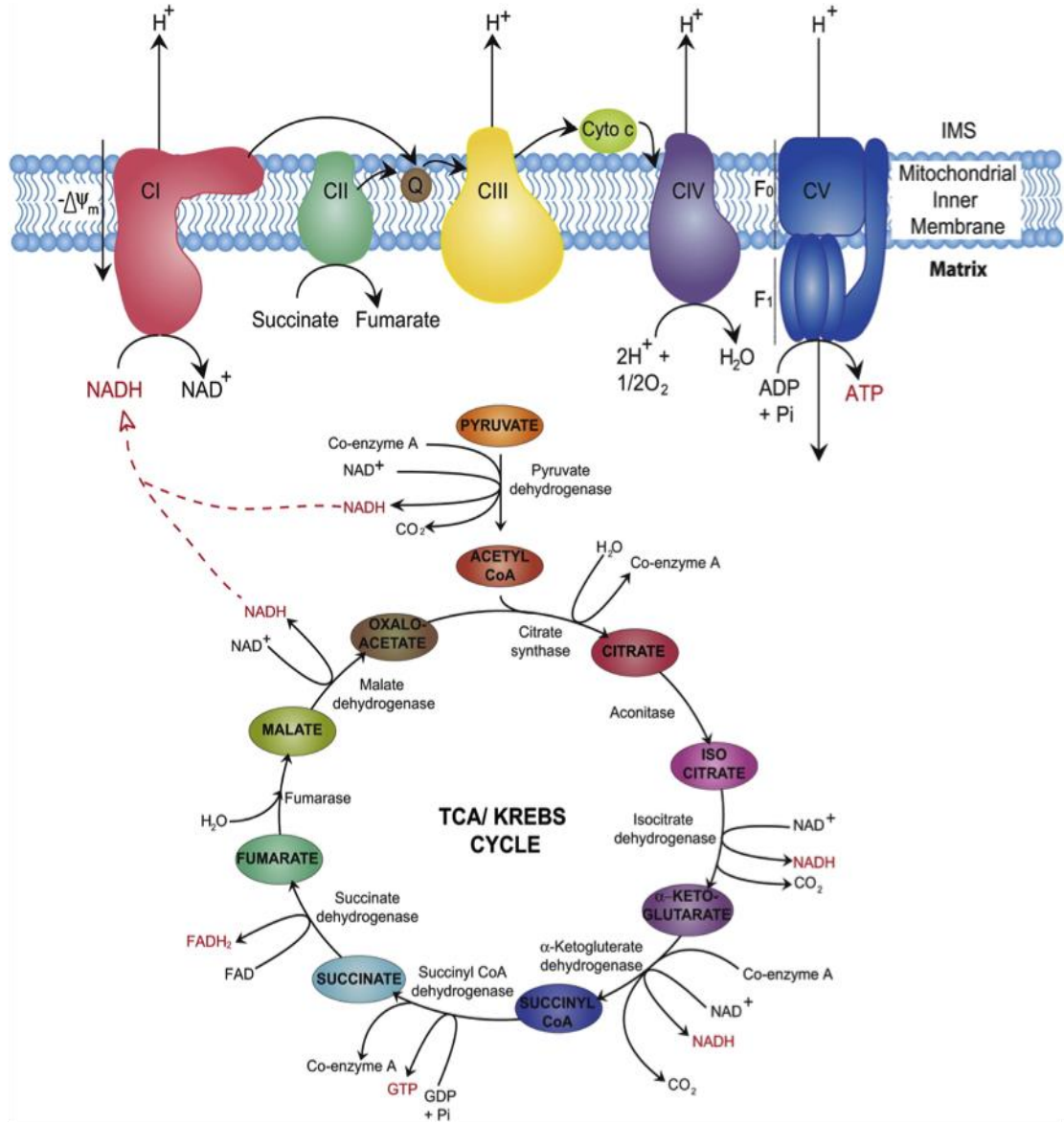
1.3.3 Lipid metabolism

Multiple groups of lipid are present but triacylglycerols are the most prevalent and consist of three individual fatty acids linked to a molecule of glycerol (Frayn 2013). Due to their hydrophobic nature they are carried in a lipoprotein throughout the bloodstream. Individual fatty acids are the building blocks of lipids and have a hydrophobic tail along with a polar carboxylic acid group and are carried in plasma bound to the protein albumin. Depending on the presence of double bonds in their carbon tail, they can be classed differently but in the context of metabolism they are referred to as 'non-esterified fatty acids' (NEFA). NEFA's are immediate carriers of lipid energy from storage depots to sites of oxidation and are supplied in two ways; 1) NEFA bound to albumin or 2) NEFA liberated from triacylglycerols (carried in lipoprotein particles) as the movement of triacylglycerols across endothelial cells that line the capillaries, exposes them to lipoprotein lipase. Their inability to condense easily means triacylglycerols are preferred for lipid storage; they completely exclude water and are therefore more efficient than not only NEFA but also glycogen at storing fuel (Frayn 2013).

The catabolism of triacylglycerols releases energy, as glycerol is converted into 3-phosphoglyceraldehyde (a component of the glycolytic pathway) and fatty acid molecules are transformed to acetyl-CoA which can then enter the citric acid cycle. This process is called β -oxidation which occurs in the mitochondria (Frayn 2013). 460 molecules of ATP are produced during catabolism of one triacylglycerol which is substantially more than the 36 during carbohydrate metabolism. Similar to glucose oxidation much of this energy is lost as heat.

Figure 5

Bioenergetics of the Krebs Cycle and Electron Transport Chain which occur within mitochondria. Pyruvate is converted into high energy molecules via Krebs cycle enzymes. NADH is shuttled to complex 1 and converted to NAD⁺ which drives oxidative phosphorylation. The resulting energy is used to create a proton gradient across the membrane and in the final step this energy phosphorylates ADP into ATP via complex V. Image taken from pg 713 (Osellame et al. 2012).

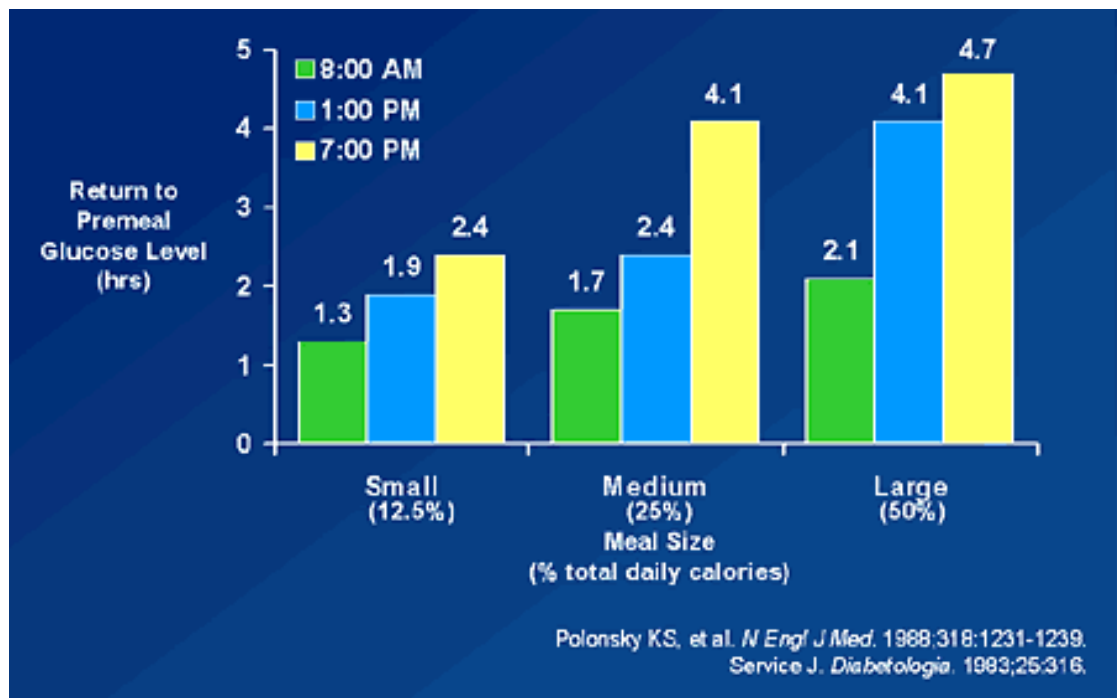


1.3.4 Fasted vs. postprandial metabolism

Although the body switches between fasted and postprandial states, with the current western diet a large proportion of the day is spent in the postprandial state. Figure 6 shows a typical Western diet in which moderate sized meals at breakfast and lunch are followed by a large meal for dinner. If this evening meal is around 50% of the total daily calorie intake it takes around 5 hours to

return to pre-meal glucose levels showing a considerable amount of time spent in the postprandial state.

Figure 6 Much of the day is spent in the postprandial state (Blonde et al. 2001)



1.3.5 Hormonal regulators

Glucose in the blood remains relatively constant despite these changing states due to the action of key regulating hormones insulin and glucagon. A small group of cells known as the *Islets of Langerhans* make up around 1-2% of the pancreas mass and are responsible for the release of these hormones. There are 3 types of cell in the islets, with the α -cells responsible for glucagon secretion and β -cells which secrete insulin and make up around 60% of the total islet volume (Frayn 2013).

The expression of the glucose transporter 2 (GLUT 2) on the β -cell membrane enables it to act as a glucose sensor and so insulin predominantly responds to plasma glucose levels (Frayn 2013). There is a sigmoid dose-response curve for the relationship between insulin secretion and glucose concentration with a steep increase in insulin when plasma glucose rises above 5mmol/L (Harrison et al. 1985). As glucose enters the β -cell and undergoes glycolysis, the production of ATP triggers a number of events which lead to exocytosis of insulin granules at the cell membrane. It's

important to note that insulin also responds to most amino acids and although an increase in fatty acids increases the insulin response, sustained increase in fatty acids can impair insulin secretion through accumulation of triacylglycerol within the β -cell (Dubois et al. 2004). Insulin travels freely in the bloodstream and binds to specific insulin receptors which are proteins consisting of two α - and two β -chains embedded in cell membranes. Once insulin binds to its receptor, tyrosine kinase activity phosphorylates β subunits causing interaction with other proteins and the beginning of a signal transduction cascade (Frayn 2013).

In contrast to insulin, glucagon's function is to increase blood glucose and it exerts its important metabolic effects on the liver alone (Frayn 2013). A rise in plasma glucose suppresses glucagon release although a rise in amino acids has the opposite effect (Frayn 2013). These pancreatic hormones are discharged into the hepatic portal vein so the liver is exposed to much higher levels of insulin and glucagon compared to other organs and it's been demonstrated that of the insulin reaching the liver, around 70% is removed (Frayn 2013).

The major organs which have a role in maintaining homeostasis during the fasting and post prandial state will be described below.

1.3.6 Metabolic tissue: Liver

The role of the liver in postprandial metabolism

The liver has a major role in energy storage in the postprandial state as it's the first organ to be exposed to nutrients entering the body from the intestine after a meal. The portal vein supplies blood to the liver which has passed through the intestinal tract.

During a postprandial state, glucose is absorbed from the intestine into the portal vein, exposing the hepatocytes to large concentrations. GLUT 2 is the glucose transporter within liver cells and because there is a high proportion of GLUT 2 and they have a high K_m (and so operate well below saturation), movement across the membrane is determined by the concentration of glucose in and outside the cell, making the liver act as a buffer (Frayn 2013).

Hexokinases which phosphorylate glucose into G6P in the liver are different from hexokinases in other tissues as they also have a high K_m for glucose and aren't inhibited by its product. Once glucose has been phosphorylated to form G6P, insulin activates the enzyme *glycogen synthase* and inhibits *glycogen phosphorylase* which results in glycogen synthesis. Storage of glucose in the liver as glycogen, enables the liver to stabilise future blood sugar so that other tissues have sufficient energy supply (mainly the brain which consumes 75% of blood glucose) (Dashty 2013). G6P can also be oxidised via glycolysis for immediate energy supply to the liver.

The liver can take up NEFA for either oxidation or storage. In the postprandial state when insulin levels are raised, storage and synthesis of hepatic lipid dominate. '*De novo lipogenesis*' (DNL) which refers to the synthesis of triacylglycerol from non-lipid precursors leads to high levels of malonyl-CoA which inhibits fatty acid entry into the mitochondria for oxidation. Fatty acids are therefore diverted towards esterification with glycerol 3-phosphate to form triacylglycerol which are stored in hepatocytes (Frayn 2013). Liver triacylglycerol therefore mainly arises from esterification of fatty acids (Perry et al. 2014).

DNL is in effect a pathway for disposing excess carbohydrate and is stimulated during high CHO availability and hyperinsulinemia. Insulin resistance which underlies many metabolic disorders raises plasma insulin levels therefore promotion of hepatic triacylglycerol storage prevails (Taylor 2012a).

The role of the liver in the fasted state

Rising levels of glucagon and catecholamines activate *glycogen phosphorylase* and inhibit *glycogen synthase* liberating glucose from stored glycogen in the liver (glycogenolysis). In addition to this, glucagon also stimulates gluconeogenesis which describes the synthesis of glucose from other precursors (Frayn 2013). Gluconeogenesis is stimulated by substrate supply so during exercise when lactate is high and during starvation when glycerol is elevated, there is an increase in gluconeogenesis. However there is a glucose paradox whereby after a meal hormones suppress

gluconeogenesis while substrate supply increases it. In the fasted state glucose enters the blood from the liver, half from gluconeogenesis and the other half from glycogenolysis (Frayn 2013).

The liver can oxidise fatty acids via the β -oxidation pathway within mitochondria which releases energy for immediate use by hepatocytes. The fasted state favours oxidation over esterification by the low insulin/glucagon ratio. Roughly 18% of each hepatocyte is made up of mitochondria (Nassir & Ibdah 2014), a vital organelle for liver metabolism being the primary site of β -oxidation and oxidative phosphorylation.

1.3.7 Metabolic tissue: Adipose

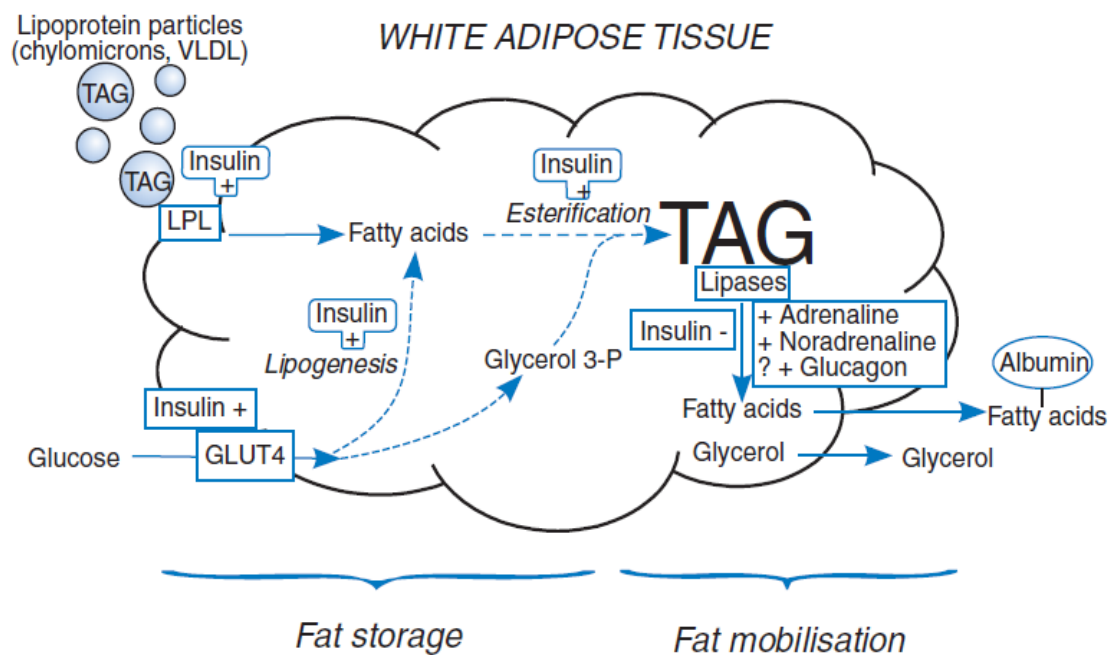
There are two types of adipose tissue; brown and white. Brown tissue is present in infants and varies across adults (Cypess et al. 2009). It has a unique metabolic feature in that it can 'uncouple' the generation of ATP from the oxidised substrates to release heat (Frayn 2013). This makes it important for energy expenditure in the form of thermogenesis and may protect against obesity although functional studies in humans are needed (Cypess et al. 2009). White tissue on the other hand is abundant in humans and controls the storage and release of fat and is therefore essential for normal health and everyday life (Frayn 2013). White adipose tissue contains multiple cell types however the focus of this review will be on adipocytes which store fat.

The role of the adipose tissue in postprandial metabolism (fat storage)

There are two sources of fat which produce the triacylglycerol droplet within adipocytes, firstly from triacylglycerols in plasma and secondly from DNL. As described earlier, triacylglycerol is transported within lipoprotein particles; these are too large to cross capillary membranes so *lipoprotein lipase* (an enzyme activated in the postprandial phase which hydrolyses triacylglycerol to release fatty acids) is present within the endothelial lining of the capillaries and acts upon passing lipoprotein particles (Frayn 2013). Physical activity, glucose and insulin all up-regulate lipoprotein lipase in adipose tissue so its activity increases in the postprandial state (Kiens et al. 1989), however in skeletal muscle it remains stable throughout the day. The beneficial role of adipose tissue is therefore evident after a meal as it protects other organs

from excess fat accumulation (Ruge et al. 2013). Fatty acids cross the interstitial space and are esterified upon entering the tissue as they are linked with glycerol-3-phosphate. Insulin stimulates both the uptake and storage of triacylglycerol as it activates lipoprotein lipase and promotes the production of glycerol-3-phosphate through glycolysis (Figure 7). DNL in adipose tissue is the same as that in the liver, stimulated by insulin to promote fat storage (Frayn 2013).

Figure 7 Adipose tissue metabolism pg 131 (Frayn 2013)



The role of the adipose tissue in the fasted state (fat mobilisation)

Lipases within adipose tissue liberate fatty acids from triacylglycerol, releasing them as NEFA bound to albumin and glycerol is also released into the plasma. This process is known as lipolysis and is catalysed by *adipose triglyceride lipase* and *hormone-sensitive lipase*. These lipases are regulated closely and inactivated rapidly in response to insulin. Insulin therefore restrains fat mobilisation as well as promoting storage (Frayn 2013). The balance between fat storage and mobilisation results in relatively stable fat stores unless long term positive energy balance pursues. In this instance, the PPAR γ and SREBP-1c systems upregulate enzymes involved in fat storage resulting in adipose hypertrophy and stimulate differentiation of pre-adipocytes into new adipocytes (hyperplasia) (Frayn 2013).

Adipose tissue also has important endocrine functions which regulate energy balance. Leptin, a protein which signals through hypothalamus receptors to restrict energy intake, is released from adipose tissue. The larger the adipose, the more leptin is produced.

1.3.8 Metabolic tissue: Skeletal muscle

Skeletal muscle requires energy for contraction, albeit in different ways. Oxidative muscle fibres (Type I) possess a high density of mitochondria and capillaries. The large supply of substrates in the blood enable these fibres to sustain muscle contraction over a long period, however diffusion of substrates into the cell requires time, resulting in relatively slow contraction. Glycolytic fibres (Type II) on the other hand have fewer mitochondria and rely on anaerobic glycolysis using G6P produced from stored glycogen within the cell. Contraction is quick but limited amount of substrates mean these fibres are important for contraction over short periods (Frayn 2013). The metabolic pathways of Type I fibres will be described below.

The role of the muscle in postprandial metabolism

Insulin stimulates glucose transporter 4 (GLUT 4) (the main glucose transporter) into action at muscle cell membranes, establishing a pathway for glucose to enter skeletal muscle. Storage of glucose pursues in the postprandial state as insulin stimulates *glycogen synthase*. 2/3 of the total body glycogen is stored within muscle cells as the total mass of muscle is large, despite there being higher levels of glycogen per unit mass of the liver (Dashty 2013). Unlike the liver, glucose cannot be released from muscle cells therefore stored glycogen is used for local sources of energy only (Dashty 2013). During muscular contraction, the breakdown of ATP stimulates glycogen breakdown; this ensures fuel is supplied to the working muscle when the energy status is low (Frayn 2013). Insulin suppresses fat mobilisation from adipose tissue resulting in low plasma NEFA concentration which causes the muscle to use glucose for fuel rather than fat (Frayn 2013).

The role of the muscle in the fasted state

The fasted state results in fat mobilisation from adipose tissue promoting the use of fatty acids as fuel for skeletal muscle. Fatty acids are taken up from

either plasma NEFA or triacylglycerol in lipoprotein particles due to the presence of *lipoprotein lipase* in capillaries surrounding muscle. The rate of fatty acid uptake depends on plasma concentration and once fatty acids are within muscle cells they can be oxidised for energy or re-esterified for triacylglycerol storage (Frayn 2013). The seamless coordination between CHO and lipid metabolism is most obvious in skeletal muscle; known as the 'glucose-fatty acid cycle' (Figure 8).

Most endogenous lipid is stored in subcutaneous and visceral adipose tissue, but a small proportion is stored within skeletal muscle- known as intramuscular lipid. Intramuscular lipid is not stored for the rest of the body, rather it's used as a readily available substrate for aerobic ATP synthesis within skeletal muscle (Frayn 2013). Intramuscular lipid can be located in either the intra or extramyocellular domains (Van Loon & Goodpaster 2006). Sedentary, obese and or Type 2 diabetes individuals have elevated intramyocellular fat, which has been linked to insulin resistance (Jacob et al. 1999). In contrast endurance trained athletes also have substantially enlarged intramyocellular fat stores but remain highly insulin sensitive. This is likely due to an adaptive response to endurance training which enables athletes to use more fat as fuel during exercise (Van Loon & Goodpaster 2006). Elevated intramyocellular fat in sedentary, obese and Type 2 diabetes patients is likely to be secondary to an imbalance between FFA availability, storage and oxidation (Van Loon & Goodpaster 2006).

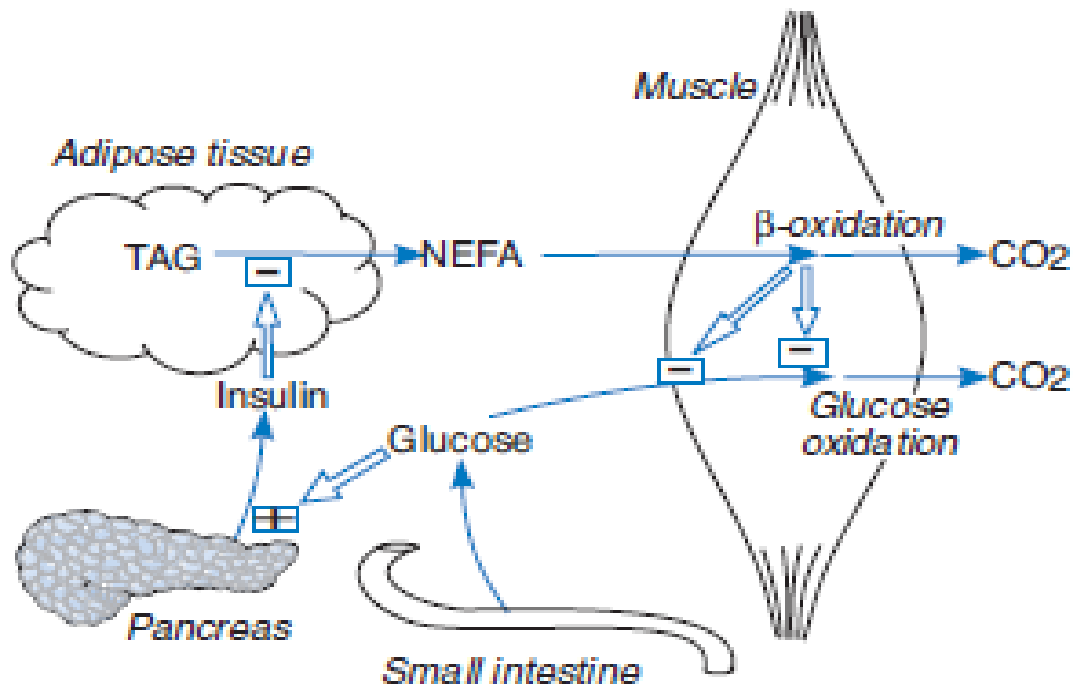
1.3.9 Metabolic interactions between glucose and fatty acids

In 1963 Randle proposed 'the glucose- fatty acid cycle' (Figure 8) which identifies the interactions between glucose and fatty acids in muscle and adipose tissue (Randle et al. 1963). Fatty acid oxidation leads to acetyl-CoA formation which generates a high rate of citrate (via the citric acid cycle). Citrate has been proposed to inhibit phosphofructokinase which is a key enzyme in glycolysis but also the generation of NADH and ATP from the citric acid cycle inhibits pyruvate dehydrogenase. The resulting effect is a reduction in glucose metabolism. The cycle may explain why several abnormalities of carbohydrate metabolism such as diabetes and obesity are associated with high levels of fat. When NEFA is elevated, operation of the

glucose-fatty acid cycle leads to impairment of glucose metabolism. Glucose membrane transport and phosphorylation of glucose is slowed which means insulin does not exert its usual effects -known as insulin resistance (Frayn 2013; Randle et al. 1963).

The competition between glucose and fatty acids has been shown to negatively influence each other's metabolism after a meal. A high fat meal the evening before an oral glucose tolerance test (OGTT-ingestion of 75g of glucose) leads to higher plasma glucose levels and similarly plasma triacylglycerols are higher during an oral fat tolerance test when preceded by a high carbohydrate evening meal (Robertson et al. 2002). This is known as the "second meal effect" and indicates that the fuel from the evening meal impairs metabolism of the alternate fuel source.

Figure 8 The glucose-fatty acid cycle , p.202 (Frayn 2013)



1.3.10 Altered metabolism-Insulin resistance

What is it?

Slight changes in the tightly controlled metabolic system can lead to significant clinical changes. Resistance to insulin-stimulated glucose uptake, known as insulin resistance (IR) is widespread and underlies many Western chronic diseases (Reaven 1988). The San Antonio Heart Study which assessed features of the metabolic syndrome in 2930 individuals found a very high degree of overlap among the six conditions linked to the metabolic syndrome (Type 2 Diabetes, hypertension, obesity, impaired glucose tolerance, hypercholesterolaemia and hypertriglyceridaemia) but the prevalence rates of these diseases in their isolated form was significantly lower (Ferrannini et al. 1991). The high degree of inter-relatedness suggests an underlying physiological network of connections. Hyperinsulinemia (raised plasma insulin which implies the presence of IR), was present in all six conditions and suggests that IR is the common denominator in which

different features (e.g. hypertension or hypertriglyceridaemia) dominate in different people (Ferrannini et al. 1991).

Pathogenesis

Ectopic accumulation of intracellular lipid in muscle and liver leads to IR, even in the absence of visceral and peripheral adiposity. The mechanism by which fat accumulates continues to be debated, but some known contributors are listed below:

- 1) Positive Calorie balance:** The commonest cause of ectopic fat is the spill-over of energy storage from adipose tissue (Shulman 2014). High fat feeding (59% fat, 20% carb) lead to significant IR after just 3 weeks in rats (Kraegen et al. 1991) and a possible explanation is that 'full' adipocytes were unable to buffer excess fatty acids leading to storage in liver and muscle cells.
- 2) Mitochondrial dysfunction:** When the rates of fatty acid uptake exceed mitochondrial fat oxidation (due to reduced mitochondrial oxidative and phosphorylation activity), there is a build-up of intramyocellular fat. This mitochondrial dysfunction is thought to be a cause of IR in the elderly (Petersen et al. 2003) and those with Type 2 diabetic parents (Petersen et al. 2004).
- 3) Dysregulated adipose:** The development of IR in only some obese individuals has supported the idea that there is an inherent component involved. The Dallas heart study (Neeland et al. 2012) followed 732 obese individuals for 7 years to identify characteristics in those who develop Type 2 diabetes. The 84 individuals who developed Type 2 diabetes displayed no differences in total body fat but significantly higher levels of visceral fat mass. It is becoming increasingly clear that obesity is not homogenous and when fat cells become 'full' they can either enter a state of hypertrophy or hyperplasia. Animal and cross sectional studies indicate that those who have a genetic predisposition to Type 2 diabetes have 'dysregulated' adipose whereby there is an inability to recruit new fat cells leading to adipose hypertrophy which is associated with inflammation (Arner et al. 2011). Problems with BMP4

and PPAR- γ signalling have been identified in the inability to recruit new fat cells (Hammarstedt et al. 2013), A consequence of dysregulated adipose is a shift of storage from subcutaneous to ectopic sites.

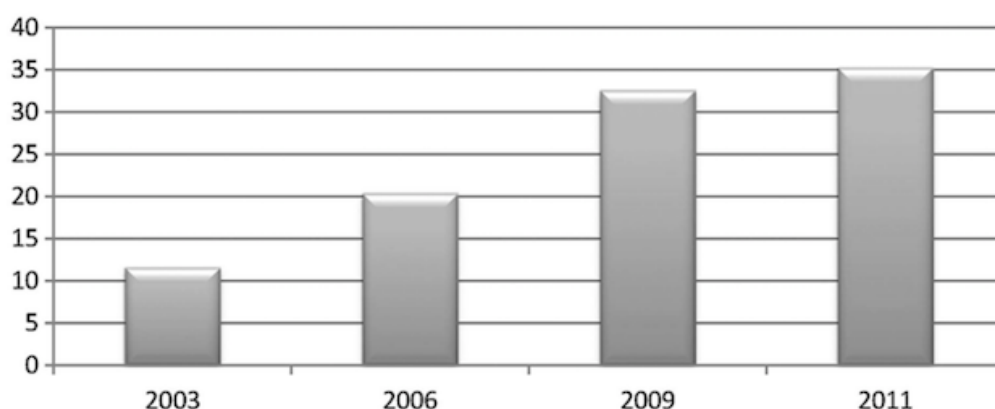
- 4) **Genetic:** Genetic causes have been identified including polymorphisms in the gene encoding apolipoprotein C3 which can predispose individuals to insulin resistance (Petersen et al. 2010) and missense mutations (I148 M in PNPLA3) which are associated with Non Alcoholic Fatty Liver Disease (NAFLD) in Hispanics (Romeo et al. 2008)

The release of diacylglycerol (DAG) from ectopic lipid is thought to be responsible for IR through activation of the theta isoform of protein kinase C (PKC θ) in muscle and the epsilon isoform of protein kinase C (PKC ϵ) in liver (Shulman 2014). Muscle tissue has two compartments of triacylglycerol, 1) Lipids as droplets in cytoplasm of muscle cells in contact with mitochondria (intramyocellular), and 2) lipids within fat cells. The increase in DAG content from intramyocellular lipid leads to increased PKC θ and phosphorylation of IRS-1. This in turn leads to decreased insulin stimulated glucose transport activity in muscle. A similar pathway occurs within the liver but increased DAG activates PKC ϵ which in turn decreases both insulin stimulated glycogen synthesis and suppression of hepatic gluconeogenesis.

Clinical presentations: Type 2 diabetes, IGT, IFG, NAFLD

IR underlies various metabolic diseases, the most common being Type 2 diabetes. There are however two conditions which precede Type 2 diabetes, known as impaired glucose tolerance (IGT) and Impaired fasting glycaemia (IFG)-both often called 'pre-diabetes'. IGT represents a stage in disordered CHO metabolism with a fasting plasma glucose $<7.0\text{mmol/l}$ and an oral OGTT 2-hour value of $\geq 7.8\text{--}\leq 11.0$ and IFG classifies a fasting plasma glucose value of $\geq 6.1\text{--}<7.0\text{mmol/l}$ (Alberti & Zimmet 1998). Data from the health survey for England revealed the prevalence rate of pre-diabetes (based on glycated haemoglobin (HbA_{1c}) of 5.7-6.4%) rose from 11.6% in 2003 to 35.3% in 2011 with those who were overweight and >40 years old having the highest prevalence rate (Figure 9) (Mainous et al. 2014).

Figure 9 Percentage of adults with pre diabetes in England by year, pg4 (Mainous et al. 2014). Vertical axis represents percentage of adult population with prediabetes, horizontal axis represents year of survey.



NAFLD

NAFLD is the commonest liver condition worldwide and is characterised by $>5\%$ intrahepatic lipid (Szczepaniak et al. 2005). Energy excess, peripheral IR and other metabolic abnormalities result in excess substrate supply to the liver, upregulation of DNL and therefore fat storage. Around 70% of patients with Type 2 diabetes have NAFLD (Targher et al. 2007), and as will be described in section 1.6.2, NAFLD plays a significant role in Type 2 diabetes aetiology.

1.4 Lifestyle behaviours and metabolism

Lifestyle behaviors that influence metabolism can be broadly categorised into sleep, movement or nutrition. Within this section we will focus on non-diet lifestyle behaviours including sleep and three distinct movement behaviours (defined below):

- 1) **Sedentary behaviour:** is defined as “*any waking behaviour characterized by an energy expenditure < 1.5 MET while in a sitting or reclining posture*” (Sedentary Behaviour Research Network 2012). It’s not just a lack of physical activity, but a distinct behavioral entity in itself. It increases cardio-metabolic risk in addition to those associated with a lack of physical activity.
- 2) **Physical activity:** is defined as “*any bodily movement produced by skeletal muscles which results in energy expenditure*” (Caspersen & Christenson 1985) . This incorporates habitual activities such as housework, gardening and walking.
- 3) **Exercise:** Exercise is a subcategory of physical activity which is repetitive, structured, planned and has a focus of improving physical fitness.

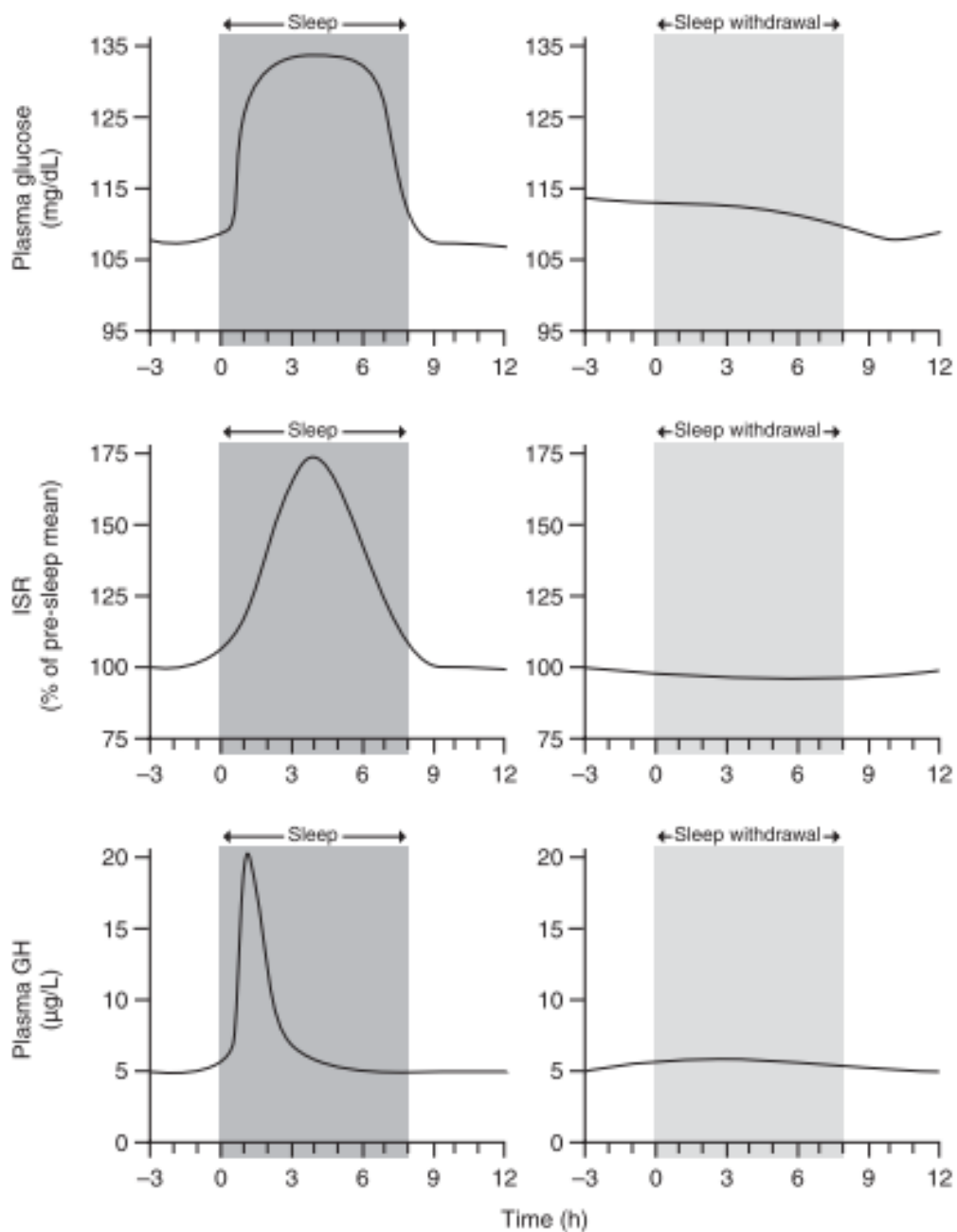
1.4.1 Sleep

Sleep is a primitive behavior, shared by all humans on a daily basis. Unlike other mammals, human sleep is generally consolidated into a single 7-9 hour period, which means an extended fasting period must be maintained. Clear physiological responses occur during sleep, including an increase in leptin levels (Simon et al. 1998), which increase satiety (Schwartz et al. 2000) and therefore reduce the drive to eat.

Sleep is a tight modulator of metabolic regulation. During sleep, when food supply is absent, the body responds by inducing a degree of peripheral insulin resistance whereby there is a marked increase in plasma glucose (20-30%) and insulin (20-30%) (Scheen et al. 1996) (Figure 10). This is important because the brain requires a continued supply of glucose despite this ‘fasting’ state. A number of factors have been attributed to this ‘insulin resistant’ state; including reduced muscle tone, low glucose requirement and

growth hormone release during slow-wave sleep (Sassin et al. 1969; Trenell et al. 2007; Boyle et al. 1994). Growth hormone is a hormone which increases blood glucose by reducing muscle uptake (Møller et al. 1991). The increase in plasma insulin during sleep is a reflection of reduced peripheral uptake.

Figure 10 Response of glucose, insulin (insulin secretion rate) and growth hormone (GH) with sleep (left panel) and sleep restriction (right panel) during constant glucose infusion. Sleep periods are shown in dark grey and sleep restriction (where sleep should be) is indicated in light grey. Image from (Trenell et al. 2007) .



The influence of sleep on metabolic regulation is highlighted in Figure 10. During sleep restriction (right panel), plasma glucose, insulin and growth hormone do not rise. Persistence of the waking condition, increases brain glucose utilisation, and growth hormone is not released, which therefore prevents the rise in plasma glucose and insulin (Van Cauter et al. 1997).

Travel across time zones, sleep restriction and shift work are all features of modern society, and changes to sleep patterns have clear influences on metabolism.

1.4.2 Sedentary behaviour

A seminal study in the 1950s reported that bus drivers who sat throughout their 5.5 hour shift had double the incidence of cardiovascular disease compared to bus conductors who were constantly performing ambulatory activities (Paffenbarger et al. 2001). This first highlighted the importance of sedentary behaviour in cardio-metabolic health.

Figure 11 Components of total energy expenditure and the energy expended from exercise in addition to non-exercise activity thermogenesis (NEAT), thermic effect of feeding (TEF) and basal metabolic rate (BMR) (Hamilton et al. 2007).

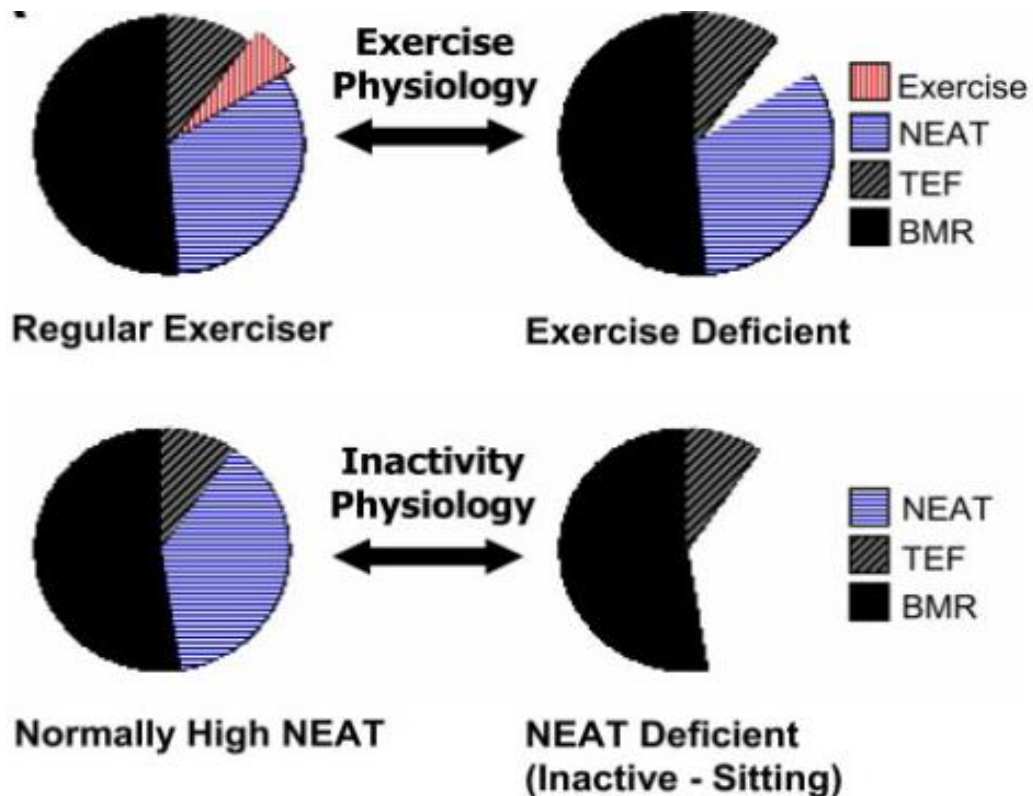


Figure 11 shows total daily energy expenditure. A currently held belief is that if a person exercises, they are considered sufficiently active. The contribution of energy expended from exercise however is very small as shown in Figure 11 and most calories are expended from Non-Exercise Activity Thermogenesis (NEAT) which includes standing and non-exercise ambulatory movements (Hamilton et al. 2007). This means that exercise, which is of shorter duration, cannot substitute for large periods of sitting time/low NEAT. Sitting for long periods reduces the number of muscular contractions, which has various physiological effects. Skeletal muscle is the largest insulin sensitive organ in the body and is responsible for 80% of insulin-stimulated glucose disposal. Immobility quickly leads to peripheral insulin resistance (Wilmot et al. 2012). In addition, lipoprotein lipase regulation is linked to local contractile activity and the decreased activity seen during sedentary behaviour leads to increased plasma triacylglycerol and reduced high density lipoprotein levels (Bey & Hamilton 2003). The differences between exercise and inactivity physiology are highlighted when measuring lipoprotein lipase, as the magnitude of lipoprotein lipase suppression after sitting is greater than the increase in lipoprotein lipase activity observed during exercise (Hamilton et al. 2007).

Increasing NEAT also has a big influence on body fat. When individuals were overfed 1000kcal/day for 2 months, those who stayed seated gained fat whereas those who increased their NEAT were able to burn off the extra calories (Levine et al. 2008).

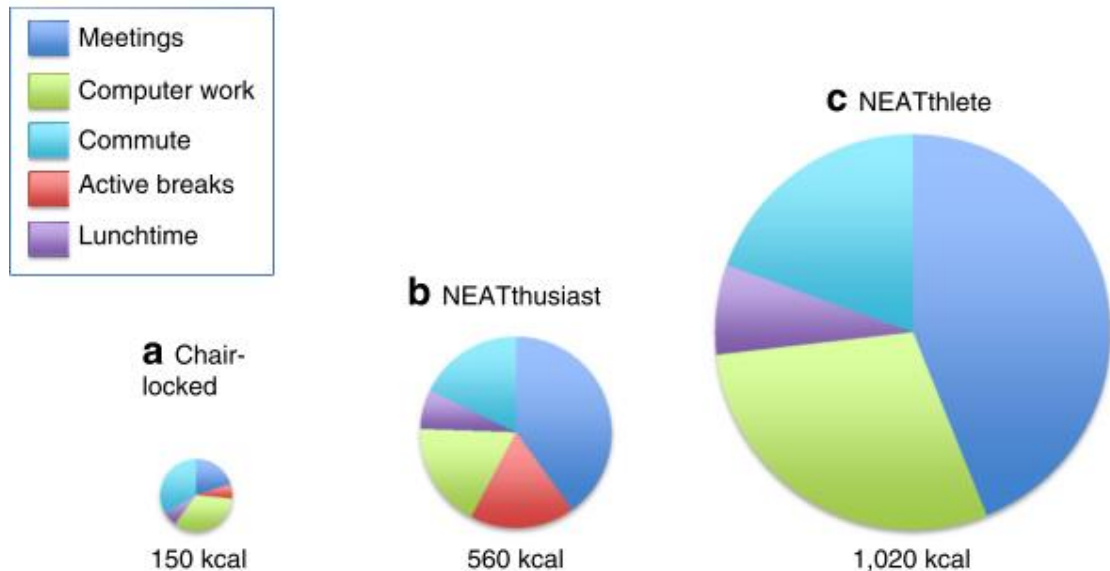
Public Health England report that more than 40% of men and 35% of women spend > 6 hours per day sitting still (Public Health England 2014a), a worrying trend which decreases the daily work performed by large skeletal muscles in the back, legs and trunk. It's likely that sedentary behaviour will increase, with continued advancements in information technology and automated devices.

1.4.3 Physical activity

One of the main benefits of physical activity is that it increases NEAT. Figure 12 displays the powerful influence of increasing NEAT on calorie expenditure.

The three pie-charts represent 3 individuals with the same desk job, but who differ in the amount of physical activity they perform daily.

Figure 12 Workplace energy expenditure for a) chair-locked worker, b) NEATthusiast who spends half of meetings walking, stroll at lunch and takes active 10min breaks, c) NEATthlete who has all walking meetings, standing desk and cycles to work (Levine 2015).



As physical activity increases energy expenditure, it reduces the risk of body fat accumulation. Evidence shows that physical activity is inversely associated with liver fat (Perseghin et al. 2007) and activity improves whole body lipid oxidation (Trenell et al. 2008). In addition, muscular contraction stimulates GLUT 4 translocation to the muscle cell membrane, thereby increasing non-insulin dependent glucose uptake. (Hayashi et al. 1997) and improving metabolic control.

Studies have demonstrated the strong link between physical activity and metabolic control. The NAVIGATOR TRIAL, a 6 year follow up study in 9306 adults with impaired glucose tolerance, found a 2000 daily increase in step count was associated with a 0.29 reduction in metabolic syndrome risk score and a 10% lower risk of cardiovascular events (Huffman et al. 2014).

The direct cost of physical inactivity in the UK is £900 million (Scarborough et al. 2011) and the health benefits of physical activity are well documented. In response, the UK government recommend adults to perform at least 150mins

of moderate or 75mins of vigorous activity over a week in bouts of >10mins (Department of health 2011a).

1.4.4 Exercise

The use of exercise in the treatment and prevention of cardio-metabolic disease did not start gaining interest until the 20th century (Moore 2004). There is now widespread evidence for the benefits of exercise, so much so, that the Centres for Disease Control and Prevention and the American College of Sports Medicine (CDC/ ACSM) suggest two default options for exercise programming. 1) Increase the patients current activity by even just a small amount, 2) Participate in large muscle group activities for 30-40mins on >4 days per week (Pate et al. 1995). A review of drug and exercise randomised trial evidence suggests that exercise is as, if not more, effective than drug interventions in the treatment and prevention of chronic diseases (Naci & Ioannidis 2013).

On a physiological level, adaptations occur during/after one exercise session (acute) or after a training programme (chronic).

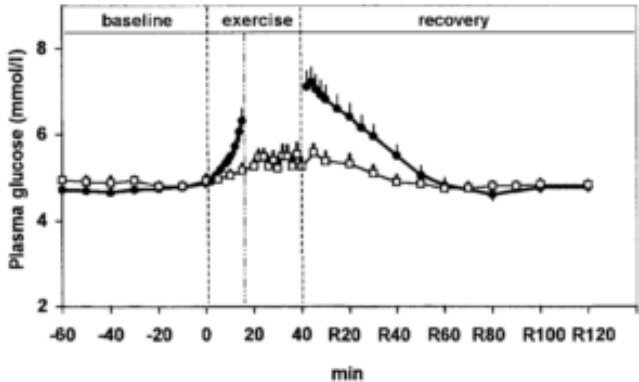
Acute

During exercise there are different metabolic responses, depending on the intensity of activity, illustrated in Figure 13. Moderate exercise maintains euglycemic homeostasis (Figure 13A) as glucose uptake matches glucose production. Sympathetic stimulation of islets and an increase in catecholamines (Figure 13C+D) leads to a reduction in insulin during exercise (Figure 13B) which increases hepatic glucose production through sensitisation to glucagon (Marliss & Vranic 2002). Glucose production during moderate exercise is therefore determined largely by the ratio of glucagon to insulin. Glucose uptake increases in muscle despite a decrease in insulin, due to exercise initiated GLUT 4 translocation and increased peripheral blood flow (Marliss & Vranic 2002).

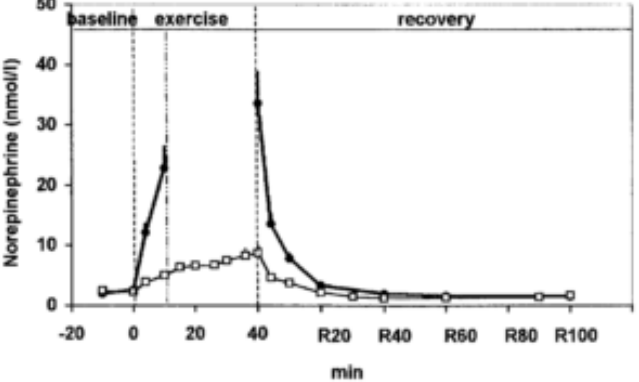
Figure 13

Metabolic responses during 40mins moderate exercise (50%VO_{2peak}-open squares) and 15min intense exercise (87% VO_{2peak}-closed circles) in young males (Marliss & Vranic 2002). A) Plasma glucose response, B) Plasma Insulin response, C) Plasma norepinephrine response, D) Plasma epinephrine response.

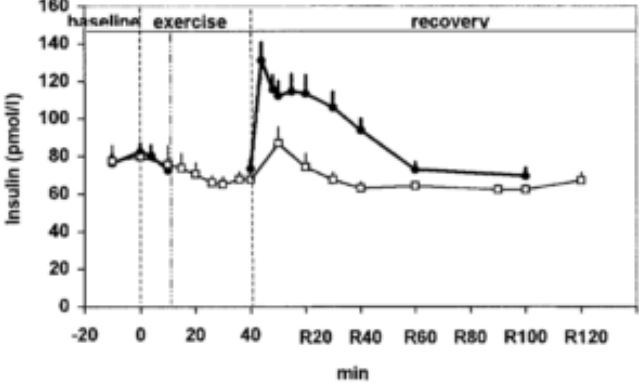
A



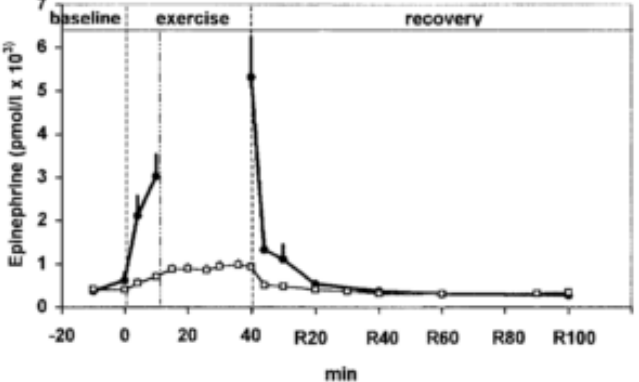
C



B



D



In comparison, intense exercise leads to a marked increase in plasma glucose (Figure 13A) as a result of rapid hepatic glycogenolysis. The large catecholamine response (Figure 13C+D) is the prime regulator of this. Despite a large increase in plasma glucose during intense exercise, insulin does not change (Figure 13B) because catecholamines prevent glucose stimulation of insulin secretion and disposal. Peripheral glucose uptake does increase but less than the increase in glucose production. Adrenergic stimulation of contracting muscle stimulates muscle glycogenolysis which therefore restrains muscle uptake of plasma glucose (Marliss & Vranic 2002). Notable changes are observed during recovery, immediate reduction in catecholamines (Figure 13C+D) leads to a rapid increase in insulin and subsequent rapid replenishment of muscle glycogen (Marliss & Vranic 2002).

Following both intensities of exercise, the main response is a short term glucose lowering effect (around 20hrs) due to the activation of skeletal muscle glucose transport (Henriksen 2002). During or immediately after exercise, plasma triacylglycerol levels rise due to increased lipolysis or remain unchanged. When a meal is given ~12 after exercise, postprandial lipaemia is reduced and the main reason attributed to this is increased lipoprotein lipase activity which peaks 4-18hours post exercise (Malkova et al. 1999). Studies looking at postprandial lipaemia after long term exercise training found no effects on plasma triacylglycerol when measured >60hours after final exercise session (Herd et al. 2000), implicating transient effects of exercise and the need to do regular activity to control plasma triacylglycerol levels.

Chronic

Numerous adaptations occur after exercise training. One known benefit is the increased capacity of the heart to deliver blood and therefore oxygen to working muscles, these central adaptations are one of the reasons for improved endurance capacity after exercise training (Holloszy & Coyle 1984).

Peripheral adaptations such as an increased capillary density and mitochondrial content also contribute to the improved endurance capacity (Holloszy & Coyle 1984). Exercise training leads to improved skeletal muscle

insulin sensitivity and glucose transport and this has been attributed to faster glycogen synthesis, up-regulation of GLUT 4 protein expression (Hughes et al. 1993) and other proteins involved in the insulin signalling cascade (Houmard et al. 1999).

Other adaptations to exercise training include; increased capacity for fat oxidation (Holloszy & Coyle 1984) improvements in blood pressure and reductions in whole body fat mass (Després et al. 1991).

There are many types of exercise training but one that has gained much attention recently is high intensity intermittent training (HIIT).

1.4.5 High intensity intermittent training

HIIT refers to brief intervals of vigorous activity interspersed with periods of low activity or rest (Gibala et al. 2012) and addresses one of the commonest barriers to exercise participation- lack of time (Trost et al. 2002). Low volume and high intensity requires a substantially lower time commitment but demonstrates comparable (if not better) physiological outcomes to moderate intensity continuous training (Gibala et al. 2012). One of the first recorded studies in HIIT was undertaken in 1972 when cardiac rehabilitation patients underwent interval training, which included 60 seconds at a high work load interspersed with 30 second rest periods. Compared to continuous cycling, patients were able to cycle twice as long after interval cycling training (Smodlaka 1972). HIIT must not be confused with 'sprint interval training', which involves 'supramaximal / all out efforts'. HIIT on the other hand includes intense activity which is submaximal (around 80-100% of maximal heart rate (Weston et al. 2013). It has recently been shown that HIIT is more enjoyable than continuous training, in inactive individuals, and promotes self-efficacy (Bartlett et al. 2011)

Acute

During HIIT there is a significant increase in catecholamines, growth hormones, blood glucose, blood lactate and initially a depletion in PCr and ATP stores, followed by a decrease in glycogen stores (Boutcher 2011). Energy expenditure and the respiratory exchange ratio (RER) increase,

reflecting a greater reliance on CHO as fuel. This is succeeded by a dramatic drop in RER after exercise (Kelly et al. 2013), potentially reflecting an elevated catecholamine response to HIIT which stimulates lipolysis and fat oxidation (Mulla et al. 2000). Oxygen uptake is elevated following HIIT, known as 'exercise post-oxygen consumption' (EPOC), to help restore metabolic process to baseline (Larsen et al. 2013). This is important because EPOC leads to increased energy expenditure and increased lipid oxidation, appetite suppression and a large hormonal response in which catecholamine's drive lipolysis, leading to abdominal and whole body fat loss (Gillen et al. 2013).

Chronic

HIIT training improves insulin sensitivity. In sedentary young males, 6 sessions of HIIT significantly reduced glucose area-under-the-curve (AUC), insulin AUC in response to an OGTT and improved insulin sensitivity (Babraj et al. 2009). After just 1 week of HIIT, there was a 20% increase in skeletal muscle GLUT 4 levels which is an important regulator of insulin sensitivity (Burgomaster et al. 2007). The increase in GLUT 4 protein alone cannot explain all of the increase in insulin sensitivity after a HIIT programme. Compared to moderate intensity exercise, HIIT requires activation of a large muscle mass and high glycogen-breakdown turnover which alters the architecture of the glycogen pool and likely affects insulin sensitivity (Calder 1991). Another benefit of HIIT is the reduction in plasma NEFA concentration (Babraj et al. 2009), and it's been shown during an OGTT that lowering of NEFA positively regulates insulin sensitivity (Santomauro et al. 1999).

There are many other peripheral adaptations including an increase in peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha (PGC1- α) (Little et al. 2011a), a key regulator of mitochondrial biogenesis in muscle (Wu et al. 1999). This increase in mitochondrial capacity proposes widespread health benefits of HIIT as PGC1- α increases oxidative capacity, glucose uptake, anti-oxidant defence and anti-inflammatory pathways. In addition, just 2 weeks of HIIT enhances maximal activity of enzymes involved

in the β oxidation pathway such as CoA dehydrogenase (32%) and citrate synthase (20%) (Talanian et al. 2007) which increases fat oxidation.

1.4.6 Measuring movement behaviours

For assessment of habitual levels and evaluation of interventions, researchers need to be able to measure movement behaviours. There are multiple methods with different pros and cons.

Indirect calorimetry

Gas exchange is considered to be the gold standard for the estimation of energy expenditure. This technique measures oxygen consumption and carbon dioxide production and using various equations can predict energy expenditure during that period of assessment (Simonson & DeFronzo 1990). The weir equation is a commonly used equation: $VO_2(3.941) + VCO_2(1.11) \times 1.44$ (Weir 1948). Energy substrates produce different amounts of energy (kcal) and the Weir equation relies on the notion that oxidation of different energy substrates is associated with a specific oxygen consumption and carbon dioxide production. The amount of oxygen and carbon dioxide during breathing therefore reflects the energy source being used and consequently energy expenditure. The mouthpiece often causes hyperventilation which overestimates values and the mask is not appropriate to wear for long periods of time (Simonson & DeFronzo 1990). In addition, direct and indirect calorimetry measurement is not practical for everyday living, unlike heart rate, questionnaires, pedometers and accelerometers which can be used for habitual living.

Direct calorimetry

The oxidation of fuel releases heat, and this heat generated by the body is measured within an insulated environment during direct calorimetry (Simonson & DeFronzo 1990). This technique has disadvantages in that it requires individuals to remain within a confined environment for long periods, requires expensive equipment, and it cannot measure evaporative heat loss (Simonson & DeFronzo 1990).

Doubly labelled water

This is an isotopic dilution method. When a human is given a loading dose of isotopes ^2H (deuterium) and ^{18}O , ^{18}O is eliminated as carbon dioxide and water, while deuterium is eliminated from the body only as water. The difference between the two elimination rates gives carbon dioxide production and therefore energy expenditure (Butler et al. 2004). Despite the high cost, need for expertise, and invasive nature, this method allows accurate measurement over 14 days has been validated against gas exchange (Schoeller & Webb 1984).

Heart rate monitors

These are widely used to measure physical activity and exercise, using the concept that an increase in heart rate reflects an increase in energy expenditure. Heart rate monitors are frequently used in exercise interventions however they are not solely responsive to an increase in work rate but rather stress, gender, the environment and hydration levels can effect readings (Crouter et al. 2004) . This compromises their accuracy in predicting energy expenditure.

Questionnaires

Self-report physical activity questionnaires have been widely used over the past 40 years. They are useful in measuring population-wide activity and the international physical activity questionnaire (IPAQ), has been shown to collect reliable and valid physical activity data in many countries (Craig et al. 2003). Despite this, there are often low correlations between scores from different questionnaires as well as objective measures such as accelerometers. The reasons for this vary, but socio-economic status, over reporting and type of activity influence the outcome (Sabia et al. 2014). Despite their limited reliability and validity compared to laboratory measures, questionnaires serve a useful purpose when measuring activity on a large scale.

Pedometers

Pedometers are a very useful, cheap tool for measuring step count and physical activity guidelines have utilised these devices to recommend an achievable target of 10,000 daily steps for health (Tudor-Locke & Bassett 2004). They measure steps by 3 methods which are; a spring-suspended horizontal lever arm, a horizontal beam and piezoelectric crystal, and a glass-enclosed magnetic reed proximity switch (Crouter et al. 2003). Pedometers also predict energy expenditure but their validity is low because they cannot detect arm activity, walking uphill, stair climbing, cycling or pushing/carrying objects (Crouter et al. 2003). They are also attenuated by tilt or impact and only count movement past a certain threshold (Bouten et al. 1997).

Accelerometers

Accelerometers measure acceleration forces and convert these into movement counts providing information on the intensity and frequency of movement. Their growing use is based on the strong relationship between energy expenditure and accelerometer output (Bouten et al. 1997).

Accelerometers can measure movement in a single axis (uniaxial) or multiple axis' (triaxial, bidirectional), with multi-axis devices have slightly higher validity scores (Trost et al. 2005). The low association between questionnaires and objective measures, along with the growing affordability of accelerometer devices make them more attractive for movement measurement (Sabia et al. 2014). Despite this, their validity does depend on the type of activity being performed as they cannot detect load carriage, changes in surface or terrain or upper body movement (Hendelman et al. 2000) and they are subject to motion artefacts.

1.4.7 Measuring body composition

Lifestyle behaviours can influence metabolic control through changes in body composition. It is therefore important to measure body composition, of which there are multiple techniques, none of which are perfect and all are subject to various constraints.

Anthropometry

Anthropometric measurements are simple, safe and cost effective surrogates for assessing obesity and body fat distribution. BMI is a simple index of weight-for-height, recommended by the World Health Organisation to classify overweight and obesity. BMI provides a useful population-level measure but it does not correspond to the same degree of fatness in different individuals, (WHO 2015b). BMI could be viewed as a measure of nutritional status, not body composition. Waist circumference and waist:hip ratio have been found to correlate strongly with BMI, and also to predict health risks (Lean et al. 1995). Waist circumference reflects the proportion of body fat located centrally, and may be more predictive of adverse outcomes than total fat, in addition to being simple to use (Wells & Fewtrell 2006). Despite their relative ease of use, BMI, waist:hip ratio and waist circumference cannot distinguish between fat and lean mass.

Skin fold thickness

Skin fold thickness gives an estimate of subcutaneous fat, and is used to predict total body fat. As the percentage of subcutaneous tissue to total fat varies across different regions, this needs to be accounted for using equations. Although this technique is relatively simple and cheap, its accuracy in individuals is poor, and it has a low inter-rater reliability and should be performed by trained staff (Ayvaz 2011). Prediction equations are often only valid in populations from which they were derived and so for indices of regional fatness across populations, it is better to leave skinfolds in raw form rather than applying predictive equations for total body fat. (Wells & Fewtrell 2006).

Bioelectric impedance

This technique measures the impedance to a small electric current as it passes through the body, and using predictive equations it calculates total body water, fat free mass and fat mass. This technique is non-invasive, quick and cheap compared to other measures of fat free mass, it is therefore often used as an epidemiological technique. However, equations are population

specific and should not be used across different groups (Dehghan & Merchant 2008).

Dual energy x-ray absorptiometry

This technique is primarily used to measure bone mineral density by measuring the absorption of 2 x-ray beams, after subtracting soft tissue absorption. As overlying soft tissue is quantified, values of fat and fat free mass are calculated using specific algorithms. DEXA is quick and provides useful information on limb lean mass (Wells & Fewtrell 2006). That being said, substantial prediction is involved for trunk measurements, bias has been noted across age, fatness and disease states (Williams et al. 2006) and this technique exposes individuals to small amounts of radiation.

Densitometry

This approach requires measurement of total body density and can measure fat mass and fat free mass by assuming specific densities of these two tissues. Total body volume is measured to allow calculation of total body density based on archimedes principle. Hydrodensitometry is considered the “gold standard” (Ayvaz 2011) in body composition analysis but due to it’s cumbersome nature, air displacement plethysmography is more commonly used (see section 3.5 for further details on this technique). Densitometry is less accurate when the composition of lean mass may be abnormal (Wells & Fewtrell 2006) but it has been shown to be reliable (Noreen & Lemon 2006).

Magnetic resonance imaging (MRI)

MRI estimates the volume rather than mass of adipose tissue (for a more detailed description of how this technique works, refer to section 1.5.3). It is very good at estimating regional body composition including subcutaneous, intra-abdominal, visceral and intramuscular adipose tissue, and there is no x-ray exposure (Wells & Fewtrell 2006). That being said, it is expensive meaning it has limited availability in the research setting. Magnetic resonance spectroscopy (MRS) is another MRI technique which can measure liver, cardiac and skeletal muscle fat through analysing the chemical composition of these tissues.

1.5 Cardiac structure function and metabolism

In simple terms, the heart is a muscular pump with two main functions; 1) Collect blood from the lungs and pump it to the body and 2) Collect blood from the body and pump it to the lungs.

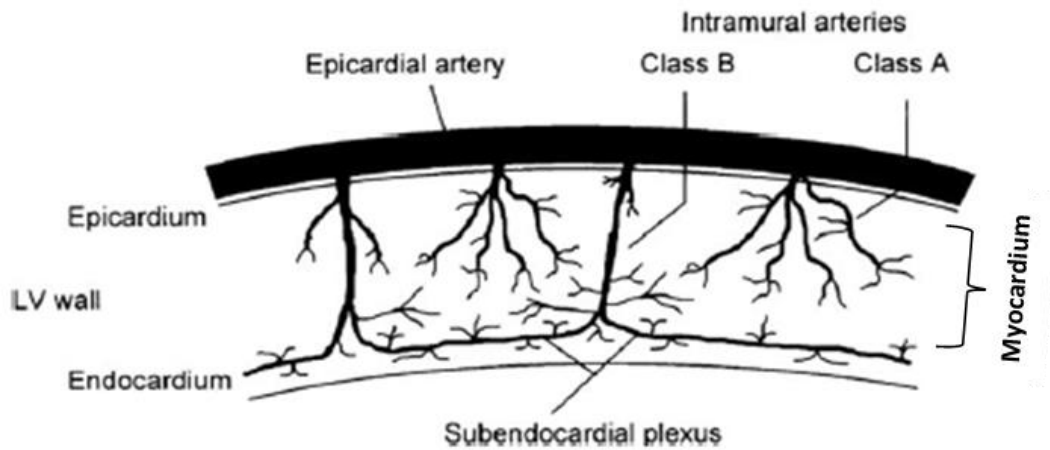
1.5.1 Cardiac structure

The heart has 4 chambers; 2 atria which collect blood and 2 stronger ventricles which pump blood around the body. Valves between the chambers ensure one way of blood through the organ.

The left ventricle receives blood from the left atrium and pumps it to all the tissues of the body whereas the right ventricle receives blood from the right atrium and pumps it to the pulmonary circulation. The left ventricle has larger muscular walls in comparison to the right ventricle as it needs to generate enough pressure to overcome resistance from the systemic circulation which is around 4 times greater than that of the pulmonary circulation (Iaizzo 2009a).

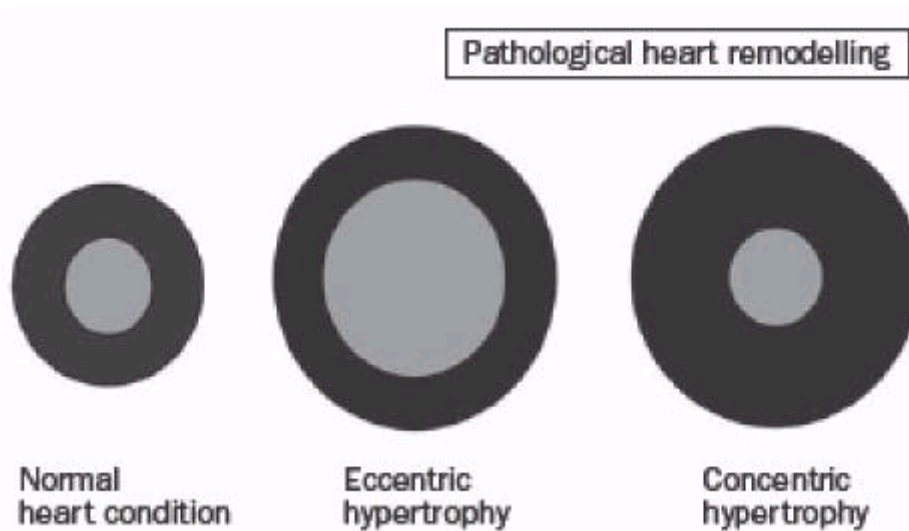
There are multiple layers to the left ventricular wall; 1) The endocardium is the internal lining which consists of endothelium tissue that rests upon elastic and collagen fibres of connective tissue, 2) The myocardium is the tissue which contracts and consists of multiple muscle cells and 3) the epicardium covers the superficial surface of the myocardium (Iaizzo 2009b). The myocardium requires a constant supply of carbon substrates and oxygen for energy during ventricular contraction. These substrates are supplied by coronary arteries, which penetrate the epicardium and supply blood to the myocardium (Figure 14).

Figure 14 Blood supply to layers of the left ventricular wall, p.300 (Iaizzo 2009b).



Various structural parameters are obtained from cardiac MRI. These include; Left ventricular wall mass (g), wall thickness at diastole and systole (mm), eccentricity ratio (g/ml), and end-diastolic/systolic blood volume (ml). 'End-diastolic volume' is the volume of blood in a ventricle at the end of its filling phase, typically 150ml in a supine healthy man. 'End-systolic volume' is the volume of blood in the ventricle at the end of contraction, around 50ml in a healthy man.(Levick 2010). The eccentricity ratio is the ratio between left ventricular mass to the end-diastolic blood volume and is a measure of concentric remodelling (Figure 15). Concentric remodelling is a pathological response to stress signals and describes the build up of collagen and increase in wall thickness which compromises end diastolic blood volume (Frey et al. 2004). This is not the case with eccentric hypertrophy, a physiological response to growth signals seen after exercise (Frey et al. 2004).

Figure 15 Pathological (concentric hypertrophy) and physiological (eccentric) heart remodelling, p.130 (Mihl et al. 2008)



1.5.2 Cardiac function

There are two main phases during cardiac contraction, systole and diastole. Systole represents the phase where blood is ejected from the ventricles, and diastole represents filling of the ventricles which lasts for around two thirds of the cardiac cycle (Levick 2010). To measure these phases using MRI, left ventricular volume is acquired for multiple phases during the cardiac cycle. Volume is calculated by multiplying the section thickness by the area of the left ventricle. This is plotted against time to produce the standard curve output (Figure 16). End-systole is defined as the lowest volume and end-diastole as the greatest volume (Kudelka et al. 1997).

Systole

The main measure of systolic function is 'cardiac output' which represents the volume of blood ejected by the ventricles in one minute. In a resting healthy man, cardiac output is around 5 L/min so that around two-thirds of the end-diastolic blood is ejected. This ejected volume is called 'stroke volume', around 70-80ml, and the proportion ejected, known as the 'ejection fraction', is the stroke volume divided by the end diastolic volume and averages 0.67 at rest. (Levick 2010).

Blood pressure is the force applied on arterial walls during the cardiac cycle. 'Systolic blood pressure' is the highest pressure in the arteries during systole and 'diastolic blood pressure' is the lowest pressure during diastole.

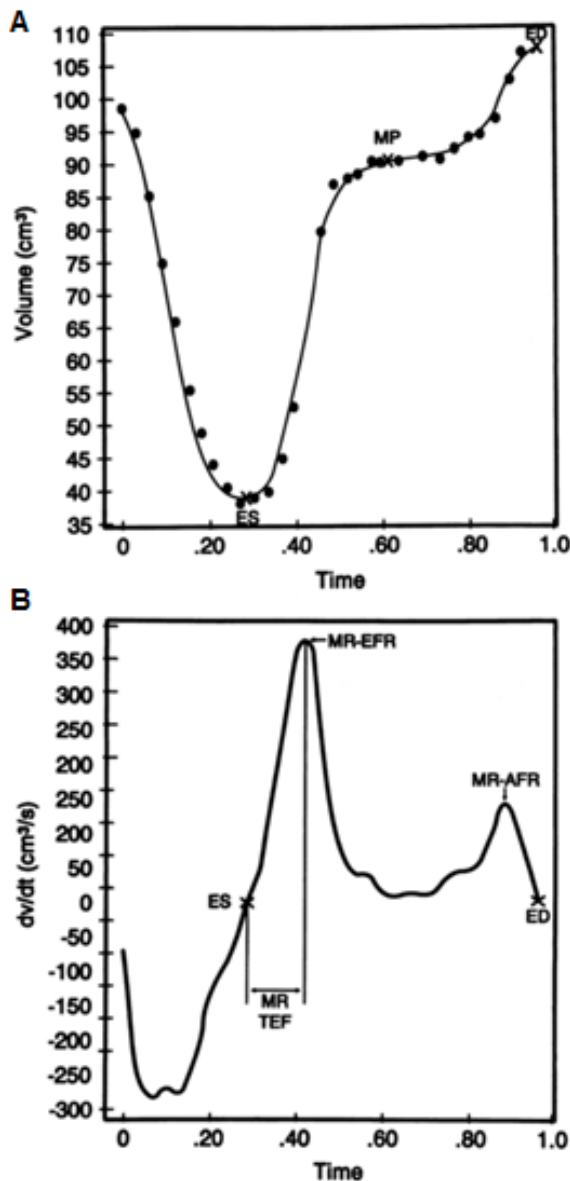
Diastole

Diastole has two phases, the early and late filling phase. Figure 16B below demonstrates this with two blood volume peaks after end-systole. The first peak represents early filling as the ventricles relax and recoil elastically from the deformed end-systolic shape, therefore sucking blood into the chamber. The second smaller peak represents late filling as the atrial muscle contract to push the remaining blood into the left ventricle. The midpoint between these two phases is called 'diastasis' which represents the period in which ventricular filling slows down and further filling is driven by venous pressure. (Levick 2010)

We can derive 5 parameters of diastolic function from cine MRI measurement.

- 1) **Early filling percentage:** The volume increase from end-systole to midpoint divided by stroke volume x100
- 2) **Peak early filling rate:** maximum value of the first derivative between end-systole and the diastolic midpoint.
- 3) **Peak late filling rate:** maximum value of the first derivative between the diastolic midpoint and end-diastole
- 4) **Time to peak early filling:** time interval between end-systole and peak early filling
- 5) **Early-to-late diastolic filling ratio (E/A):** peak early rate divided by the peak late rate E/A is the first generation test for diastolic performance. If the ratio is >1 then diastolic function is considered 'normal' but any results <1 represents diastolic dysfunction (Kudelka et al. 1997).

Figure 16 Left ventricular volume during a cardiac cycle from MRI in a healthy volunteer. A)-spline fitted curve (Time is expressed as a fraction of the RR interval).B) First derivative of the curve shown in A. ES=End systole, MP=diastolic midpoint, ED=end-diastole, MR-AFR= peak late filling rate, MR-EFR= peak early filling rate, MR-TEF= Time to peak early filling (Kudelka et al. 1997).



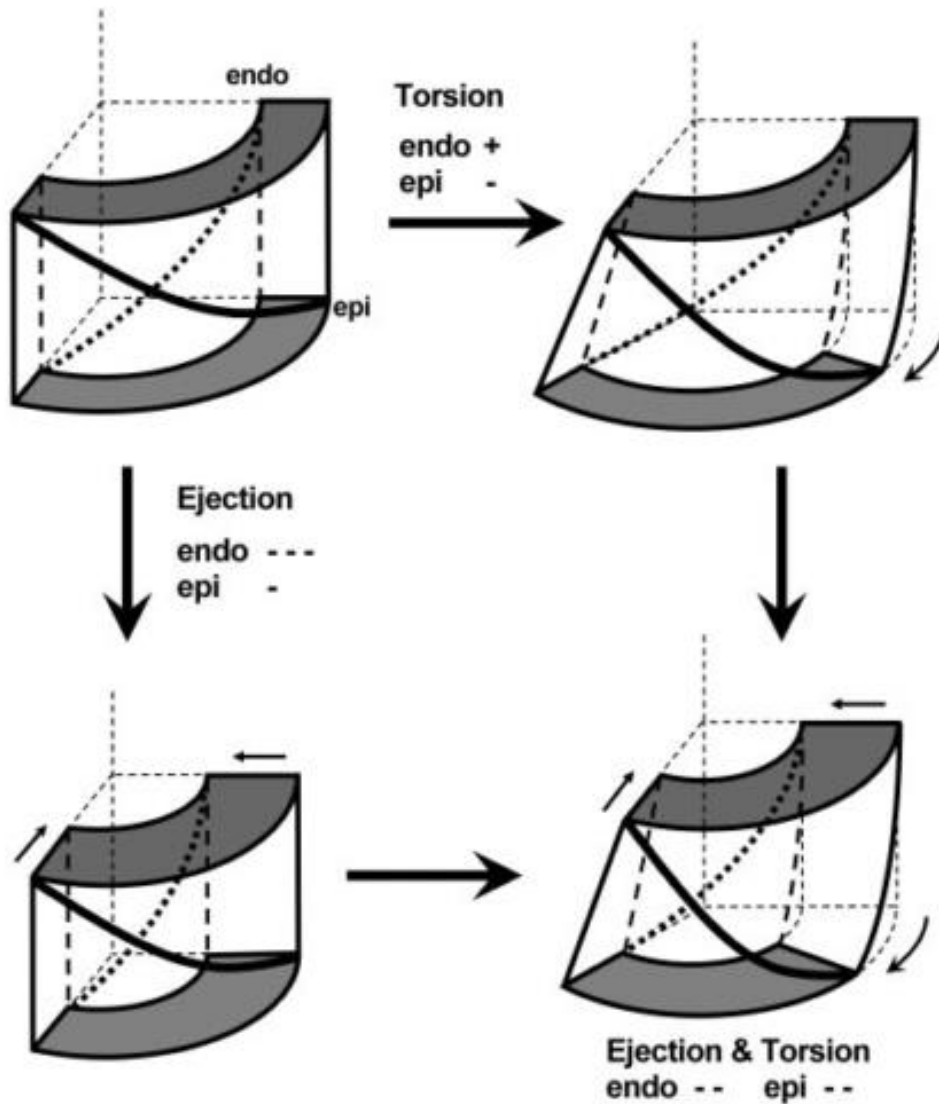
Torsion and strain

Torsion is a normal feature of cardiac contraction and describes the relative rotation of the apex with respect to the base in a counter-clockwise direction (Buchalter et al. 1990). This twisting motion occurs as epicardial fibres are further than endocardial fibres from the centre of the left ventricle and therefore have a mechanical advantage. The endocardial fibres therefore partly counteract torsional motion induced by the epicardium (Lumens et al. 2006) and so damage to endocardial fibres seen in ageing and various

diseases, causes net torsion to increase (Hollingsworth et al. 2012; Fonseca et al. 2004). During contraction, the myocardium displays two modes of action: shortening (ejection) and torsion (Figure 17). The ratio between these two is called the torsion to shortening ratio (TSR) and is seen to increase in normal ageing due to endocardial fibre damage (Lumens et al. 2006). During early diastole, torsion is rapidly released and this is reported as the torsion recoil rate which is normalised for peak torsion (%/ms) (Hollingsworth et al. 2012).

Regional torsion angle ($\Delta\Theta$) is the angle between a superior (basal) tag point at end-systole and the corresponding tag point on an inferior slice, expressed as an angle of rotation (Buchalter et al. 1990) (Figure 18). Cardiac Torsion is the circumferential longitudinal shear angle (γ) which refers to the difference between a tag point on a basal and inferior slice at end systole (Figure 18) expressed as an angle of which the corner is the tag point on the basal slice (Buchalter et al. 1990). The 'Circumferential longitudinal shear' (γ) is calculated using the regional torsion angle and the radius of the myocardial border (r). There is regional variation in torsion, in that torsion increases towards the base of the heart (Buchalter et al. 1990).

Figure 17 Modes of action during left ventricular contraction, from a segment of the myocardium. *Top right* shows torsion in the absence of ejection, *bottom left* shows ejection in the absence of torsion. *Bottom right* shows a normal physiological state, p.1574 (Lumens et al. 2006)



In addition to torsion, strain is also a feature of myocardial segment deformation, which refers to stretching or compression in 3 directions (Figure 19). These measures are affected by wall thickness and have been shown to correlate strongly to ejection fraction (Bogaert et al. 2001). Left ventricular longitudinal shortening, a parameter which is calculated from MRI, is a measure of the percentage change in length of the left ventricle in a longitudinal direction (Petersen et al. 2011a).

Figure 18 The torsion angle ($\Delta\theta$) and circumferential longitudinal shear (γ) for one tag point, p.1239 (Buchalter et al. 1990).

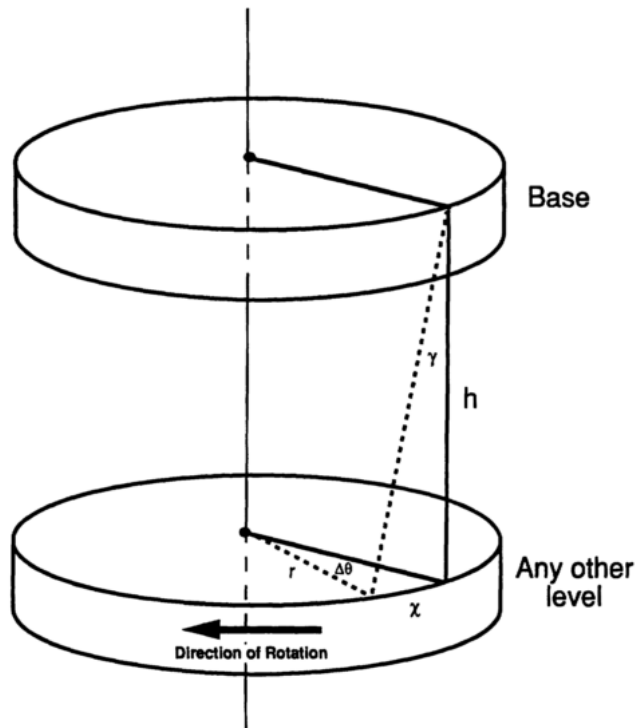
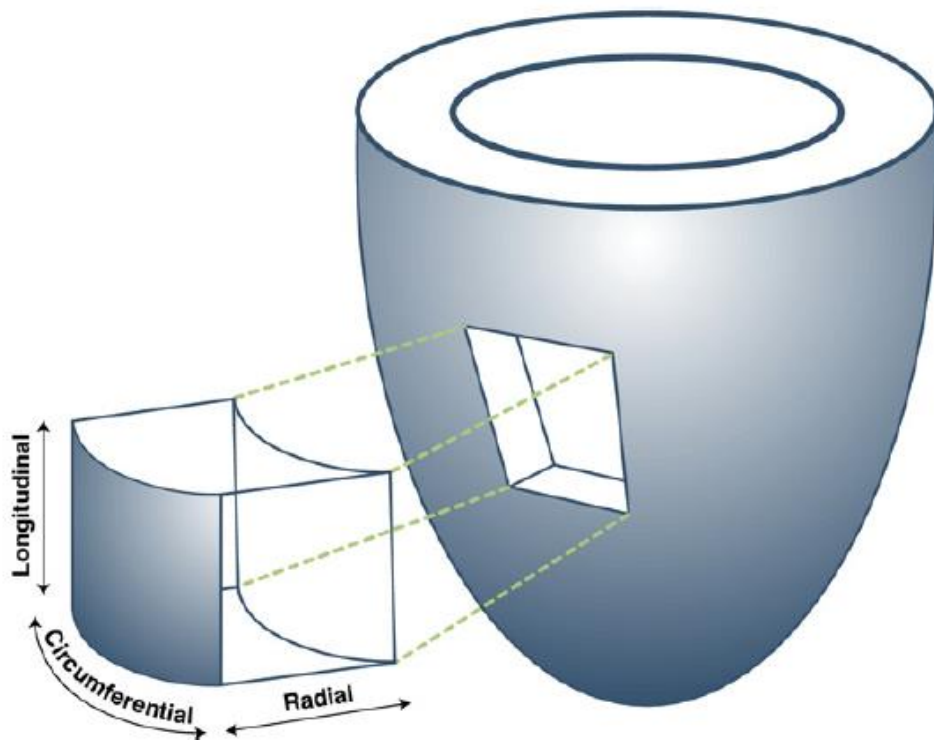


Figure 19 Three types of myocardial strain, p.674 (Petersen et al. 2011b)



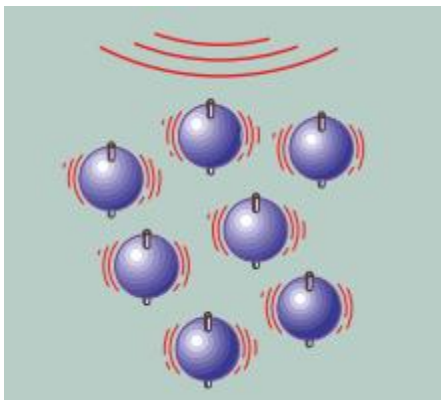
1.5.3 Cardiac Magnetic Resonance Imaging

Measures of left ventricular structure and function have high clinical and diagnostic value and are commonly used in risk assessment and therapeutic decisions (Haider et al. 1998). Echocardiography is widely used to measure the left ventricle but this technique relies on geometric assumptions (Missouris et al. 1996). MRI is superior in that it provides a spatially defined 3-dimensional dataset so no geometric assumptions are made and is therefore regarded as the gold standard for left ventricular, mass, volume and some functional measurements (Plein et al. 2001; laizzo 2009b).

How it works

The body's natural magnetic properties are used in MRI to produce images. Hydrogen atoms, used because of their abundance in water and fat, behave like a small bar magnet as they spin with their axes randomly aligned. A 3.0 Tesla scanner provides a strong magnetic field causing alignment of the proton axes and creation of a magnetic vector orientated along the scanner axis (Figure 20) (Berger 2002).

Figure 20 Magnetic field causing the proton axes to line up, p.35 (Berger 2002)

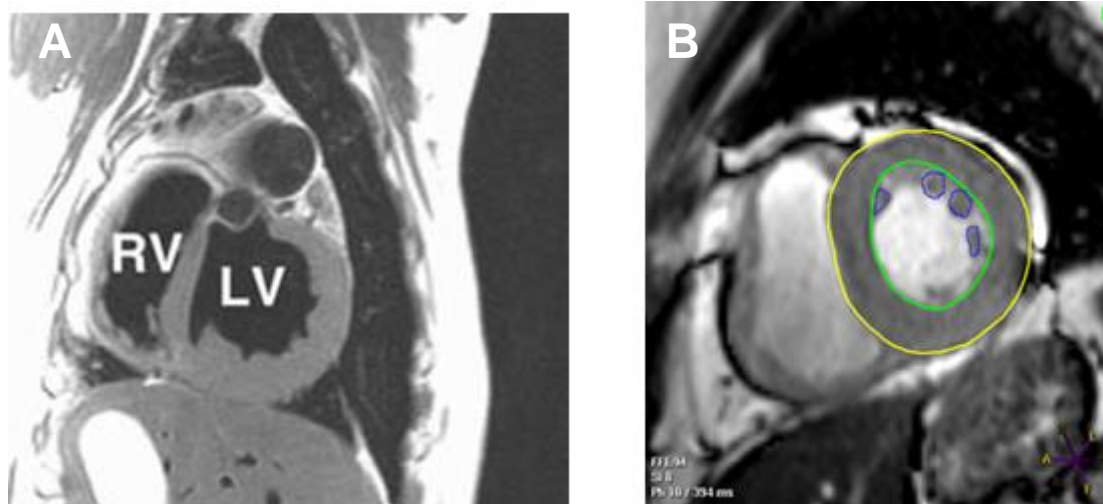


Radio-waves are then applied which deflects the magnetic vector, then switching off of the radiowave causes the vector to return to its resting state, emitting a signal. This signal is used to create MRI images. Different tissues can be detected because they relax at various rates. Relaxation is measured in two ways; 1) T1- time taken for the magnetic vector to return to its resting state, 2) T2-Time taken for the axial spin to return to resting. Receiver coils act as aerials to improve detection of signals (Berger 2002). To reduce

movement artefact, acquisitions are synced with heart rate thereby minimising cardiac motion and breath holds are required to remove respiratory movement (Fuster et al. 2001).

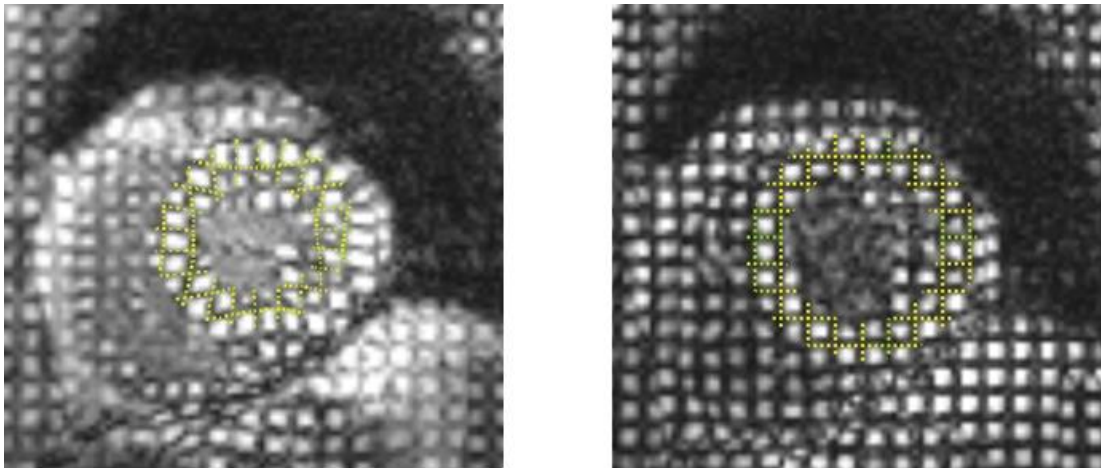
To measure cardiac structure a 'spin echo' sequence is used which creates 'black-blood' images due to the signal void created by flowing blood (Figure 21A), enabling good contrast between the blood and myocardium (Iaizzo 2009b). These images allow the calculation of structural and functional parameters obtained by tracing around the endocardial and epicardial borders of multiple short axis slice images of the left ventricle (Figure 21B). Ventricular volumes are then calculated using 'Simpons rule' (approximates area under the curve).

Figure 21 Cine MRI A) Spin echo imaging showing the black cavities of the left and right ventricle and the surrounding myocardium. p.345(Iaizzo 2009b). B) Manual tracing of epicardial and endocardial borders.



Tagging is used to measure myocardial strain and torsion. A series of pulses null the longitudinal magnetisation along thin strips, which appear as tags. These are applied in two directions to form a grid pattern. These tags imbed in the tissue and distort during myocardial motion. This can be used to trace motion of the myocardial wall, as tags within the ventricular blood disappear quickly due to the motion of blood (Iaizzo 2009b) (Figure 22).

Figure 22 Left ventricular tags at end systole (left) and end diastole (right).



1.5.4 Cardiac metabolism

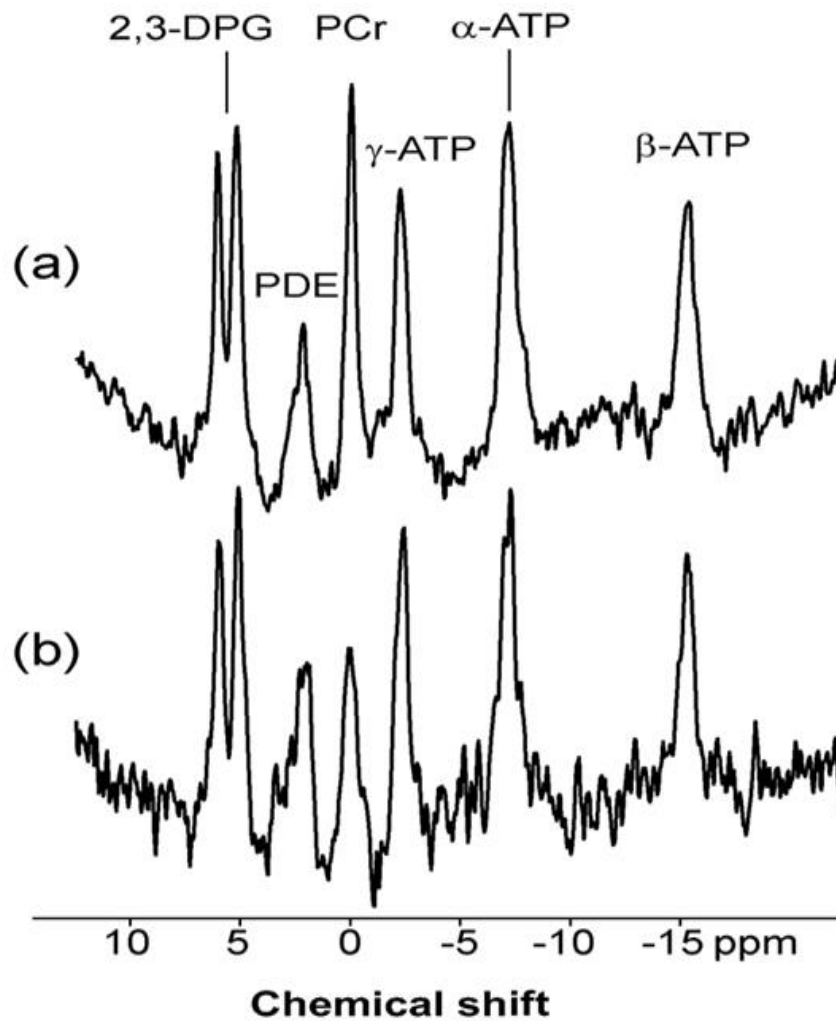
The heart is metabolically active, using around 6kg of ATP each day to enable roughly 100,000 heart beats daily (Bizino et al. 2014). Both diastole and systole require energy and to fuel this requirement, the heart converts free fatty acids and glucose into chemical energy in a ratio of 3:1 respectively (Bizino et al. 2014). Fatty acids are derived from either plasma NEFA or triacylglycerol as the heart expresses high levels of lipoprotein lipase (Frayn 2013). Fatty acid uptake by sarcolemmal fatty acid transport proteins consumes energy and enters the mitochondrion where β -oxidation takes place. Glucose uptake by GLUT 4 is insulin dependent and is converted into pyruvate before entering the Krebs cycle in the mitochondrion (Bizino et al. 2014). The glucose-fatty acid cycle operates in the myocardium so that under fed conditions when insulin is high, glucose is utilised over fat (Frayn 2013). The heart can also metabolise pyruvate, lactate and ketone bodies but their low blood concentrations means this is rare. When all the blood concentrations of substrates are equal, the heart favours fatty acid, pyruvate and lactate metabolites (Iaizzo 2009b).

1.5.5 Cardiac Phosphorus Magnetic Resonance Spectroscopy (P-MRS)

Radiofrequency pulses used during MRI, can be used for MRS. The signal however is not used to create images, but rather used to measure the content of MR-visible nuclei including Hydrogen (^1H), Carbon (^{13}C), and phosphorus (^{31}P). P-MRS is used to estimate the bioenergetics state of the cardiac tissue by measuring the phosphocreatine/ATP ratio (PCr/ATP). MRS

can also be used to measure intrahepatic and intramuscular liver lipid using H-MRS (Befroy & Shulman 2011). Figure 23 shows an example cardiac spectra, there are multiple peaks because they need slightly different magnetic fields to bring them to resonance at a particular radiowave. The ratio of the areas under the peaks represent the number of atoms in each environment (rather than the height).

Figure 23 Sample cardiac phosphorus spectra from (a) a young subject (with PCr/ATP = 1.95) and (b) an older subject (with PCr/ATP = 1.55).



1.6 Type 2 diabetes

1.6.1 Definition and diagnosis

Type 2 diabetes is a metabolic disorder characterised by hyperglycaemia due to disorders of insulin secretion and insulin action or both (Alberti & Zimmet 1998). Despite having these hallmark insulin disorders, Type 2 diabetes is polygenic and heterogenic in nature (McGarry 2002).

Diagnosis of this chronic disease is built upon a biochemical threshold which has changed over the years. In 2011, the WHO confirmed an HbA_{1c} value of >48mmol/mol (6.5%) as the primary diagnostic criteria for Type 2 diabetes but emphasised a value <48mmol/mol doesn't exclude diagnosis using other glucose tests. The progression of normoglycemia (fasting venous plasma glucose of <6.1mmol/l) to Type 2 diabetes occurs over time with IGT and IFG frequently preceding Type 2 diabetes. For example the Baltimore longitudinal study of aging which examined Caucasian 21-96 year olds over a long period, found that compared to those with normal glucose levels at baseline, those who had IFG or IGT experienced higher progression rates to Type 2 diabetes 5 years on (Meigs et al. 2003). Although this suggests a slow transition in glucose control-more recent data suggests 18 months prior to Type 2 diabetes diagnosis, there is an abrupt rise in fasting glucose (Sattar et al. 2007a). This is likely to reflect an underlying metabolic change during the pathogenesis of Type 2 diabetes and emphasises the need for 6/12monthly checks in people who have IGT/IFG.

1.6.2 Pathogenesis: Twin cycle hypothesis

In the past, Type 2 diabetes was thought to be a chronic, progressive condition however reversal of Type 2 diabetes after gastric surgery (Dixon et al. 2008) and dietary intervention (Lim et al. 2011) has challenged this belief and provided insight into the pathogenesis of the disease. The normalisation of blood glucose and liver fat after these led to the 'twin cycle hypotheses' and belief that Type 2 diabetes is a disease of chronic excess fat.

Liver fat

What-Reversal of Type 2 diabetes during a very low calorie diet and gastric surgery is characterised by a rapid decrease in liver fat. After only 7 days of a 600-kcal/day diet, liver fat fell by 30% along with a reduction in fasting plasma glucose from 9.2mmol/L to 5.9mmol/L (Lim et al. 2011). More evidence to suggest that liver fat is central in the pathogenesis of Type 2 diabetes can be found with the rise in alanine aminotransferase (ALT) prior to Type 2 diabetes diagnosis (Sattar et al. 2007b) which indicates metabolic stress in hepatocytes.

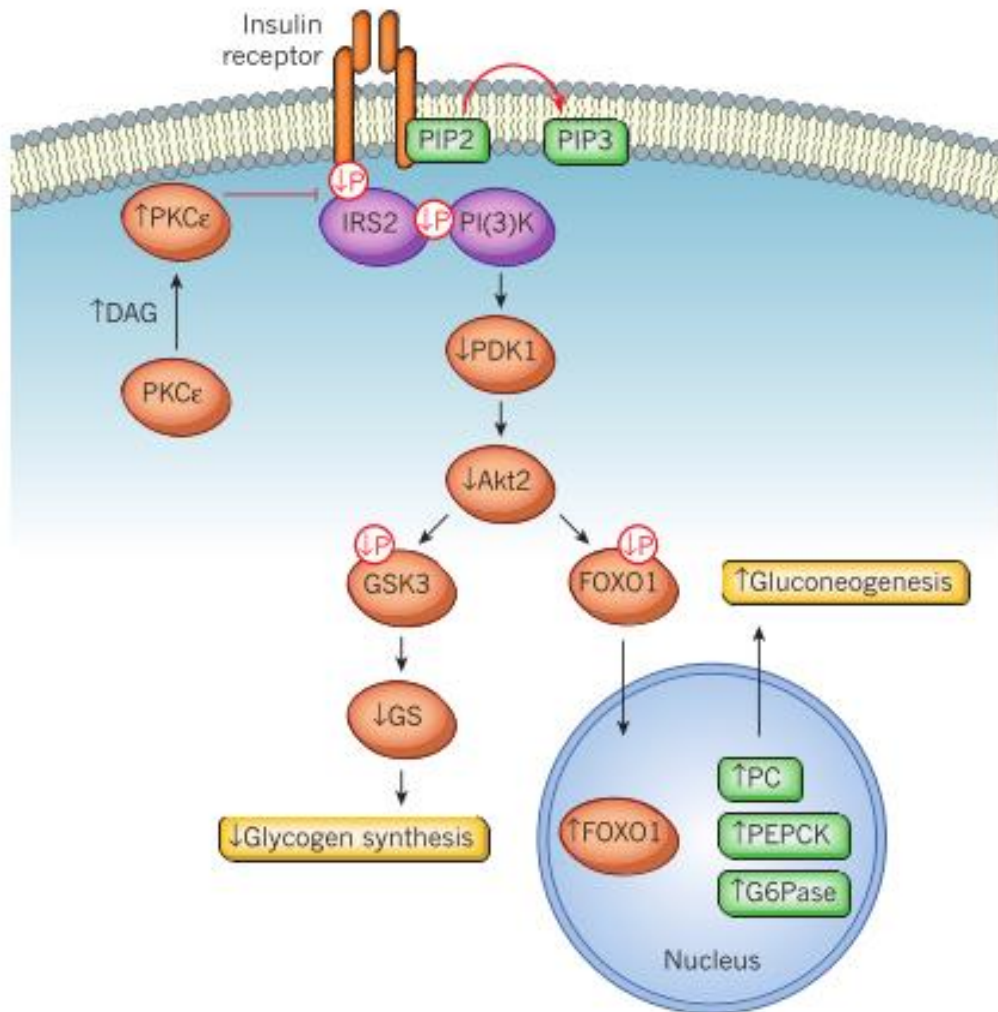
Why-Chronic positive energy balance results in conversion of CHO into fat via DNL. Once glycogen depots are full, excess CHO can only be converted into triacylglycerol within the hepatocytes, and this *in situ* conversion is favourably stored rather than transported to adipose tissue (Taylor 2012b). 3 weeks of carbohydrate overfeeding in the form of sweets, 300ml of pepsi and 30ml fruit juice, led to a 30% increase in liver fat (Sevastianova et al. 2012).

Along with energy excess, hyperinsulinemia also leads to intra-hepatic triacylglycerol deposition. Those who have peripheral IR as a result of lifestyle or familial traits, have raised plasma insulin levels, and fat accumulation in the liver persists because insulin stimulates DNL (Taylor 2012b). One study showed that 70% of people with Type 2 diabetes have a fatty liver (Targher et al. 2007)

Consequences-Liver fat is responsible for causing hepatic IR and consequently the inability of hepatocytes to respond to insulin leads to continued endogenous glucose production (Seppala-Lindroos 2002). Liver fat is linked to hepatic IR as the accumulation of DAG (a product of DNL) leads to activation of protein kinase-C ϵ (PKC ϵ) which inhibits the insulin signalling pathway (Figure 24) (Perry et al. 2014; Samuel et al. 2010). The fall in liver fat during surgery or a very low calorie diet occurs alongside a fall in plasma glucose because of normalisation of hepatic insulin sensitivity (Ravikumar et al. 2008).

Figure 24

Molecular pathway in which DAG leads to hepatic IR and hyperglycemia (Perry et al. 2014)



Although metabolically one of the fates of newly synthesised triacylglycerol is oxidation in mitochondria for energy production, a by-product of DNL called malonyl-CoA inhibits fatty acid transport into the mitochondria (Taylor 2013). This results in hepatic triacylglycerol being directed to exportation as very-low-density-lipoprotein (VLDL) levels or storage in hepatocytes.

B-cell dysfunction

What-A main characteristic of Type 2 diabetes is insulin deficiency due to β -cell dysfunction. Being the organ which regulates insulin production, changes in the pancreas are seen during Type 2 diabetes and we now know that β -cell number is around 40% less at the time of Type 2 diabetes diagnosis with

a continued linear deterioration thereafter (Rahier et al. 2008; Butler et al. 2003)

Why-Normally, an increase in blood glucose stimulates β -cell insulin release due to ATP generation by glucose oxidation. However in Type 2 diabetes increased NEFA, according to the Randle cycle (Randle 1998), leads to a decrease in glucose metabolism, or in other words β -cells become less responsive to glucose. In addition, the long term exposure of β -cells to hyperglycaemia is partly responsible for the decline in β -cell function as it causes the β -cells to become unresponsive, even in non-diabetic individuals (Ferner et al. 1986). When human islets are exposed to high levels of glucose and NEFA over 48hours, there is a decrease in insulin content and a loss of glucose stimulated insulin secretion, mimicking the physiological β -cell changes that occur in *vitro* (Dubois et al. 2004). This suggests that a high level of glucose isn't a prerequisite for β -cell changes with elevated NEFA but it is interesting to note that when both conditions are present, their additive effects worsen β -cell deterioration. These metabolic factors account for the acute decrease in insulin secretion seen in Type 2 diabetes.

The loss of total β -cell mass observed in Type 2 diabetes has been attributed to chronic exposure to fatty acids and deposition of VLDL leading to apoptosis (Shimabukuro et al. 1998). When human islets are exposed for 48 hours to a fatty concentration of that which mimics a Type 2 diabetic patient, there is a significant increase in triacylglycerol accumulation and β -cell apoptosis (Dubois et al. 2004).

Consequences-As already stated; the main consequence is a decrease in insulin secretion and β -cell apoptosis. However it's becoming clearer that there isn't a universal fat threshold which determines β -cell dysfunction but rather individuals have different degrees of liposusceptibility (Tushuizen et al. 2007; Taylor 2013)

The COUNTERPOINT study (Lim et al. 2011) illustrated that first phase insulin response was absent in Type 2 diabetes patients (diagnosis <4yrs) but after 8 weeks of a very low calorie diet (VLCD) this returned to normal and the insulin secretion rate was no different to non-diabetic individuals.

This suggests that β -cells dysfunction is not permanent and should be viewed as 'metabolic inhibition' rather than complete β -cell destruction (Taylor 2013). Whether this applies to individuals with a longer Type 2 diabetes diagnosis is still to be determined.

Twin cycle

The twin cycle hypothesis suggests these steps operate as a 'twin cycle' rather than a linear sequence (Figure 25) (Taylor 2012b).

'Liver fat cycle' refers to the raised liver fat which increases hepatic IR and therefore fails to inhibit endogenous glucose production. This continued production stimulates insulin release which further drives DNL, increasing hepatic triacylglycerol content.

'Pancreas Cycle' points to the increased fat and glucose production which cause β -cell dysfunction leading to reduced insulin secretion and therefore further elevations in plasma glucose.

1.6.3 Altered metabolism

During normal metabolic regulation the body acts to 'buffer' the entry of substrates into the circulation; plasma glucose remains relatively constant at around 5 mmol/L and can rise to around 8 mmol/L but any further rise is prevented via an increased clearance mainly into skeletal muscle and suppression of endogenous glucose production. Similarly lipid levels are controlled after a meal with increased triacylglycerol clearance mainly into adipose tissue and suppression of endogenous triacylglycerol into the circulation (Frayn 2013). This metabolic regulation is orchestrated by insulin, which has specific effects on different tissues. Disruption of this finely coordinated system can be attributed largely to IR which is the earliest detectable defect in Type 2 diabetes (Petersen et al. 2012) and its affects are illustrated in Table 1.

Insulin stimulated glucose uptake is reduced to the same extent in patients with IGT, IFG and Type 2 diabetes therefore IR by itself cannot account for the differences in glucose tolerance deterioration between pre-diabetes and diabetes (G M Reaven 1988). The ability of β -cells to compensate for the

defect in insulin action determines glucose tolerance (G M Reaven 1988). In response to IR, insulin secretion is elevated which can be sustained in obese individuals who never progress to Type 2 diabetes (Frayn 2013).

Figure 25 The twin cycle hypothesis of Type 2 diabetes (Taylor 2013)

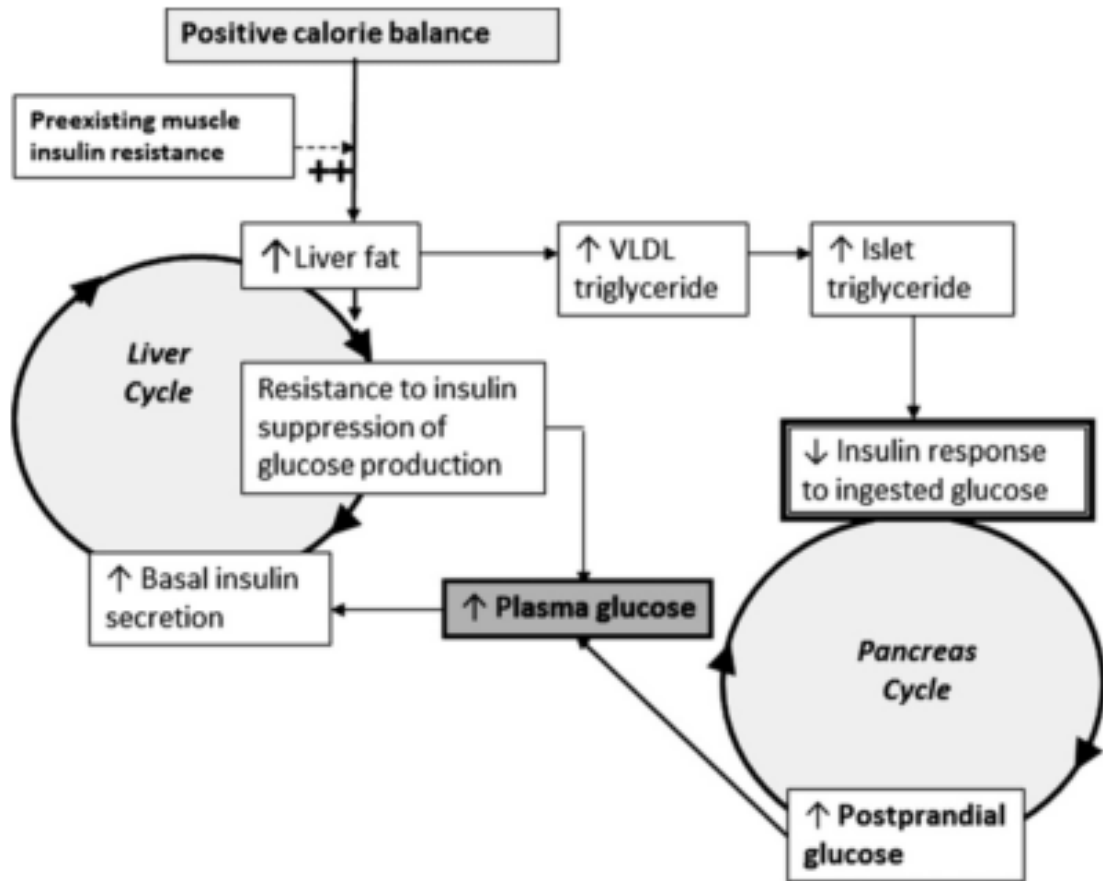


Table 1 **The impact of insulin and insulin resistance on major organs**

Organ/Tissue	Insulin (working normally)	Insulin Resistance
Liver	Inhibits gluconeogenesis Inhibits glycogenolysis Stores glucose Inhibits the release of VLDL	Continued release of glucose into blood High levels of plasma insulin stimulate DNL
White adipose tissue	Inhibits lipolysis Take glucose out of the blood and store it as fat	Keep releasing fat into the blood Inhibits the tissue from taking glucose out of the blood
Muscle	Take glucose out of the blood and stores it as glycogen Stimulates glycolysis/glucose oxidation for energy Stop using fat for energy	Inhibits the tissue from taking glucose from the blood Store fat
Brain	Stop eating	Eat (or keep eating)

Glucose metabolism

Type 2 diabetes is characterised by raised plasma glucose throughout the day with exaggerated responses to meals. Challenging homeostatic mechanisms with 75g of glucose (OGTT) is an effective way of illustrating altered glucose metabolism with Type 2 diabetes. This is characterised by higher fasting glucose levels, peak glucose levels and 2-hour glucose levels after ingestion of 75g of glucose. The combination of IR and decreased β -cell insulin secretion causes reduced glucose uptake and continued production of endogenous glucose. 219 men with either normal glucose tolerance, IGT and Type 2 diabetes were challenged with 75g of glucose, and peak insulin secretion occurred at 60 mins in normal glucose tolerance, 90 mins in IGT and 120 mins in Type 2 diabetes. This delay in peak insulin secretion

manifests itself as elevated plasma glucose throughout an OGTT (Bergstrom et al. 1990).

Fat metabolism

Small differences in plasma insulin concentration profoundly influence plasma NEFA concentration (Gerald M Reaven 1988) and it's well established that fasting and postprandial plasma NEFA levels are elevated in Type 2 diabetes irrespective of whether individuals are obese or not (Fraze et al. 1985).

Figure 26 Fasting and postprandial plasma glucose, insulin and FFA concentration in 15 normal and 15 Type 2 diabetes divided into mild (FPG <140mg/dl), moderate (FPG 140-250 mg/dl) and severe (FPG >250mg/dl)(Fraze et al. 1985).

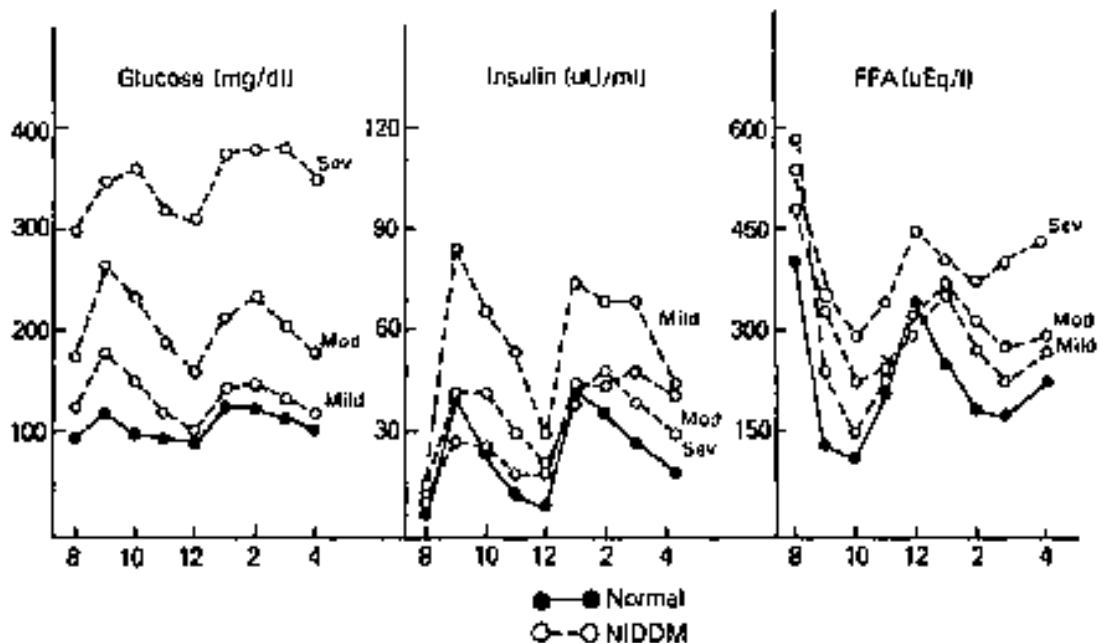


Figure 26 demonstrates that in individuals who could maintain hyperinsulinemia (classified as having mild Type 2 diabetes) maintained near normal levels of glucose and NEFA whereas those with IR who secreted insulin levels equivalent to 'normal' individuals (classified as having severe Type 2 diabetes) had a significant increase in plasma glucose and NEFA (Fraze et al. 1985).

Elevated NEFA in Type 2 diabetes is attributed to adipose tissue IR, which decreases esterification of fatty acids and increases lipolysis within adipose tissue (Fraze et al. 1985). A vicious cycle is in operation with high NEFA

concentrations because the increased flux of substrates promotes hepatic gluconeogenesis (Gerald M Reaven 1988) and according the Randle theory higher supply of NEFA to muscle prevents glucose uptake, both of which increase plasma glucose (Frayn 2013). Elevated NEFA and VLDL which are characteristic of a typical diabetic phenotype are not only a consequence of Type 2 diabetes but are also involved in the pathogenesis of this metabolic disorder (Taylor 2012b).

1.6.4 Altered lifestyle behaviours

A large observational study in females demonstrated 90% of Type 2 diabetes cases were associated with unhealthy lifestyle behaviours which include; poor diet, smoking, alcohol consumption and physical inactivity (Hu et al. 2001). The next section will review the evidence that altered non-diet lifestyle behaviours are associated with Type 2 diabetes.

Sleep and Type 2 diabetes

No study better demonstrates the modulating role of sleep in metabolic regulation than Spiegel et al. (Spiegel et al. 1999). Less than 1 week of sleep debt (4hr per night) in young healthy men led to pre-diabetes. In fact, studies consistently show a U shaped relationship between sleep duration and Type 2 diabetes risk (Shan et al. 2015). 7-8 hours sleep is associated with the lowest risk, and a significant increase in risk is observed with shorter or longer sleep. The first study to look at incident diabetes and change in sleep duration found that a 2 hour increase in sleep duration over 5 years led to increased risk of Type 2 diabetes (Ferrie et al. 2015). Although an obvious consequence of prolonged sleep is reduced time for physical activity in waking hours, it is likely that there are physiological mechanisms which underpin the relationship between long sleep and Type 2 diabetes risk, but these have yet to be elucidated.

The physiological mechanisms linking short sleep and Type 2 diabetes are more widely studied. After sleep restriction, glucose tolerance reduces by around 40% (Spiegel et al. 2004) with an increase in hepatic glucose production and decrease in peripheral glucose disposal. A number of reasons have been attributed to this, including; 1) an increase in sympathetic

tone (which decreases β -cell responsiveness and leads to inadequate pancreatic insulin secretion), 2) reduced AKT phosphorylation, 3) inflammation and 4) decreased melatonin secretion (melatonin associated with Type 2 diabetes risk) (see (Ferrie et al. 2015) for review). In addition, short sleep leads to a >70% increased ghrelin-to-leptin ratio, increasing the desire for calorie-dense foods, weight gain and resulting metabolic abnormalities (Spiegel et al. 2004).

Despite the clear link between sleep shortening/lengthening, government policies do not mention sleep as an important behavior to target in the prevention, management and treatment of Type 2 diabetes.

Sedentary behaviour and Type 2 diabetes

Sedentary behaviour is positively associated with Type 2 diabetes risk (Wilmot et al. 2012) in a dose dependant manner. (Grontved & Hu 2011). A 3 year follow-up of the US diabetes prevention programme demonstrated reduced Type 2 diabetes incidence with the lowest sedentary time (Rockette-Wagner et al. 2015). These observations are all independent of body mass index (BMI), which suggests that the impact of sitting extends beyond the effect on body composition. Despite these results, much of the evidence arises from self-report measures, focusing on television sitting time. Indeed a recent study which used daily sedentary time with accelerometers demonstrated significant associations with metabolic parameters cross-sectionally, but sedentary time did not predict 5 year diabetes incidence (Barone Gibbs et al. 2015). This highlights the need for objective measures and longer term follow to better define the relationship between diabetes and sedentary time.

In those with Type 2 diabetes, higher sedentary time is associated with larger waist circumference, homeostasis model assessment (HOMA)-IR, insulin and lower high density lipoprotein (HDL) cholesterol (Cooper et al. 2012) and intervention studies have shown that frequently breaking up sedentary time reduces postprandial glucose and insulin response (Dunstan et al. 2012). Despite this evidence, individuals with Type 2 diabetes continue to be more sedentary than their healthy counterparts (Hamer et al. 2013). Health

guidelines for Type 2 diabetes do not prioritise sitting time as a modifiable risk factor to target. Due to the barriers of performing physical activity, increasing NEAT and reducing sedentary behaviour seems a practical solution in those with Type 2 diabetes and more work needs to be done into translating this evidence into clinical practice.

Physical activity and Type 2 diabetes

A systematic review looking at moderate physical activity levels (defined as 3-6 metabolic equivalents (METS)) and Type 2 diabetes risk concluded that those who regularly engaged in moderate physical activity had a 30% lower risk of Type 2 diabetes compared to sedentary individuals (Jeon et al. 2007). This was also the case when specifically walking was examined. It is important to note that these associations remained significant when controlling for BMI (Jeon et al. 2007). In a worldwide analysis of physical inactivity and disease outcomes, it was estimated that inactivity was associated with a Type 2 diabetes risk ratio of 1.20 (Lee et al. 2012). Compared to all the chronic diseases they studied, the highest prevalence of physical inactivity occurred in those who went onto develop Type 2 diabetes.

Evidence that physical activity lowers Type 2 diabetes risk also comes from interventional studies. Two seminal studies, the Finnish (Tuomilehto et al. 2001) and US Diabetes Prevention Programmes (Knowler et al. 2002), have shown that large scale lifestyle interventions are as, if not more, effective than pharmacological interventions. The interventions encouraged individuals to reduce their body weight by >5% and increase physical activity to 150 mins per week (measured by self-report). The Finnish study which recruited 522 individuals with IGT found that the relative risk reduction in progression to Type 2 diabetes reached 43%, 7 years on (Lindström et al. 2006). A 10-year follow up of the US diabetes programme demonstrated a 34% reduction in diabetes incidence, higher than the 18% reduction with metformin (Knowler et al. 2009). A recent systematic review found that in those who are at increased risk for Type 2 diabetes, physical activity and diet interventions significantly reduce diabetes incidence and improve cardio-metabolic risk factors, compared with usual care (Balk et al. 2015). Benefits of physical

activity interventions extend to substantial financial savings, with prescription costs reduced by \$259 in one study (Di Loreto et al. 2005).

Despite all the evidence, individuals with Type 2 diabetes still do not participate in adequate levels of physical activity (Nwasuruba et al. 2007).and more work needs to be done to improve this lifestyle behaviour in cardio-metabolic disease.

Exercise and Type 2 diabetes

Along with diet and medication, exercise has been a cornerstone in the management of Type 2 diabetes for decades and its low cost and non-pharmacological nature make it an attractive therapy. The latest exercise recommendations in Type 2 diabetes are derived from the American College of Sports medicine (ACSM) and the American Diabetes Association (ADA) joint position statement in 2010 (Colberg et al. 2010).

Using the frequency, intensity, time, type (FITT) principles they recommend aerobic activity to be:

- F** performed at least 3 days/week and due to the transient nature of exercise effects on exercise, there should be no more than 2 consecutive days between bouts.
- I** Exercise should be at least moderate (40-60% peak oxygen consumption (VO_{2peak})) but additional benefits are seen for exercise >60%. Intensity may be more important than volume.
- T** Minimum of 150 mins/week. Bouts of activity should be at least 10 mins long spread throughout the week
- T** Large muscle groups which increase heart rate.

FITT principles for resistance exercise:

- F** At least twice weekly on non-consecutive days along with aerobic exercise
- I** Moderate (50% of 1 repetition maximum (RM)) to vigorous (75-80% 1RM)

- T Minimal of 5-10 exercises targeting major muscle groups and each set should include 10-15 reps progressing to heavier weights with 8-10 reps. 3-4 sets per exercise is recommended.
- T Resistance machines and free weights are both recommended.

Below I will present evidence from systematic reviews and meta-analysis regarding exercise in Type 2 diabetes.

Thomas et al.(Thomas et al. 2006) conducted a systematic review of all literature in Type 2 diabetes and exercise to explore the independent effects of different types of exercise upon this chronic condition. Only 14 randomised controlled trails (RCTs) comparing purely exercise and non-exercise with a total number of 377 participants were used, as many other trials combined exercise with weight loss. There was a significant 0.6% reduction in HbA_{1c} with the exercise intervention, which varied from 8 weeks to 12 months in duration and combined aerobic and resistance exercise. A 1% increase in HbA_{1c} is associated with a 21% increase in disease related death, 21% increase in disease end point, and a 37% increase in microvascular complications (Thomas et al. 2006) suggesting this 0.6% decrease is clinically significant. Despite significant improvements in insulin sensitivity, exercise had no effect of fasting plasma glucose or AUC for glucose, and there was no change in blood pressure or blood cholesterol after exercise. Subcutaneous and visceral fat significantly decreased with body weight remaining stable.

Similar findings were reported from a meta-analysis which included 27 exercise studies over 4 weeks to 2 year duration and included 1002 Type 2 diabetes individuals. The average reduction in HbA_{1c} was 0.8% for longer term studies, described by the authors as 'small to moderate benefits on glucose control' which were similar to other dietary, drug and insulin treatments (Snowling & Hopkins 2006).

Volume + intensity: The benefits of providing structured exercise interventions was highlighted in a meta-analysis of 47 RCTs (Umpierre et al. 2011) which showed greater improvements in glucose control compared to

just providing physical activity advice and it was also evident that the volume of exercise is important with >150 mins/week reducing HbA_{1c} by 0.89% and <150mins/week reducing HbA_{1c} by 0.36%. Another more recent systematic review and meta-analysis of supervised exercise confirmed the importance of exercise volume (Umpierre et al. 2013) and the importance of exercise intensity has also been highlighted (Boulé et al. 2003).

Type: The ACSM and ADA (Colberg et al. 2010) recommend that a combination of resistance and aerobic exercise is better than focusing on one training modality (evidence category B) in Type 2 diabetes. A meta-analysis which analysed aerobic, resistance and combined training, found combined was most effective for blood glucose, lipids and blood pressure (Schwingshackl et al. 2014). Aerobic training was superior when compared to resistance training for glycaemic control. The external validity of these findings may be questioned, as only supervised interventions were included in the analysis.

HIIT and Type 2 diabetes

Previous to 2011, no studies had looked at HIIT in Type 2 diabetes. Little et al. (Little et al. 2011a) were the first group to examine the benefits of HIIT in this metabolic condition but due to the extremely demanding Wingate-based HIIT program, they developed a more practical HIIT model which included 10 x 60s sprint intervals eliciting 90% maximal heart rate. Eight participants with Type 2 diabetes performed 6 sessions of this HIIT model and using continuous glucose monitoring they found average 24 hour blood glucose was significantly reduced 48-72 hours after the last training bout, as well as 3 hour post-prandial AUC glucose curve for breakfast, lunch and dinner. Biopsy samples from the vastus lateralis displayed an increase in mitochondrial capacity with raised electron transport chain proteins, raised citrate synthase activity (20%) and a large increase in GLUT 4 content (369%). These positive changes were observed despite a 50% lower time commitment compared to current exercise recommendations.

The acute effects of a single HIIT session (10 x 60second sprints) was compared to a control day in 7 individuals with Type 2 diabetes (Gillen et al.

2012). Postprandial glucose concentration significantly decreased after HIIT and time spent in hyperglycaemia in the 24 hour period after HIIT was reduced by 65%. Postprandial hyperglycaemia is a major contributor to Type 2 diabetes related complications and reducing post meal glucose excursions is a priority for Type 2 diabetes management (Ceriello et al. 2004).

The only longer term HIIT intervention (12 weeks) in Type 2 diabetes was undertaken in 45 Southeast Asian adults (Mitranun et al. 2014). Fasting glucose decreased from 7.7 to 6.6 mmol/L and HbA_{1c} from 60 to 54 mmol/mol. The modest improvements in glycaemic control are likely to reflect increased peripheral insulin sensitivity due to muscular adaptations highlighted by Little's group (Little et al. 2011b). South Asian populations are more susceptible to insulin resistance and Type 2 diabetes therefore the applicability of these findings to Caucasians is questioned.

In summary, the HIIT trials in Type 2 diabetes show acute improvements in glycaemic control and peripheral insulin sensitivity however the need for longer term studies using HIIT in Type 2 diabetes is warranted before HIIT can be considered an established therapy in Type 2 diabetes management.

1.6.5 Altered cardiac health

The term '**cardio-metabolic disease**' has arisen from the increased risk of cardiac complications in metabolic disease, and also from the fact that they seem to share common environmental and genetic antecedents.

The Framington heart study (Kannel & McGee 1979) was one of the first to identify an increase in cardiovascular disease (CVD) in men and women with Type 2 diabetes and identify CVD as the leading cause of mortality in Type 2 diabetes (Garcia et al. 1974). The effect of hyperglycaemia and other risk factors contributing to atherosclerotic vascular disease have been established (Miki et al. 2013). In contrast, 'diabetic cardiomyopathy' which can be defined as the dysfunction of cardiac tissue in the absence of coronary heart disease, (Larsen & Aasum 2008) has received less attention. Despite Type 2 diabetes posing at least a 2-3 fold increased risk of heart failure (Kannel et al. 1974), heart failure has been described as the 'frequent, forgotten and often fatal complication of diabetes'. (Bell 2003). A recent 6

year longitudinal study found that compared to healthy controls, prediabetes and diabetes patients had significant subclinical myocardial damage, even after adjustment for CVD risk factors. Furthermore, these people were at higher risk of heart failure and mortality (Selvin et al. 2014).

It is consistently reported that one of the earliest preclinical manifestations of cardiomyopathy is left ventricular diastolic dysfunction, which may progress to systolic dysfunction and resulting heart failure. Abnormal cardiac geometry (remodelling) has also been reported but evidence is less robust. Alterations in cardiac metabolism were thought to underlie these changes (Diamant et al. 2003), however more recent evidence suggest they are independent of each other (Rijzewijk et al. 2009). These changes are described in more detail below.

Altered cardiac structure

Cardiac MRI in middle aged to elderly men and women demonstrated a significant association between increases in the eccentricity ratio with hyperglycaemia and IR (Velagaleti et al. 2010). This finding however is not consistent; with some MRI studies reporting no difference in left ventricular wall mass between Type 2 diabetes patients and controls (Diamant et al. 2003; Rijzewijk et al. 2009).

Altered cardiac function

A common finding in asymptomatic individuals with Type 2 diabetes is left ventricular diastolic dysfunction with normal left ventricular ejection fraction. There is a high prevalence of diastolic dysfunction in asymptomatic, normotensive patients with Type 2 diabetes (Diamant et al., 2003), with one study reporting diastolic dysfunction in 43 out of 57 (75%) individuals (Boyer et al. 2004). Myocardial relaxation is an essential cardiac function. When there is a need for increased diastolic relaxation such as during exercise, healthy individuals can increase the rate of myocardial relaxation which allows for an increase in left ventricular filling, despite shortened diastolic filling time (Oh et al. 2011). A less compliant heart means relaxation (E) is reduced and atrial systole (A) contributes a greater proportion of diastolic filling (Oh et al. 2011).

Altered cardiac strain and torsion

Fonseca et al (Fonseca et al. 2004) measured strain and torsion in control and Type 2 diabetes patients. Individuals with Type 2 diabetes demonstrated significantly lower peak systolic circumferential and longitudinal strain and significantly higher peak left ventricular torsion during systole. Diastolic relaxation rates of longitudinal and circumferential strains were also lower in Type 2 diabetes individuals, which would be expected when considering the association between diastolic dysfunction and Type 2 diabetes. Despite individuals in Fonseca's study having normal ejection fraction, systolic dysfunction was manifest with a decrease in left ventricular longitudinal shortening. It seems likely that the raised torsion in Type 2 diabetes compensates for the reduced longitudinal shortening, to maintain ejection fraction (Fonseca et al. 2004). TSR is increased in Type 2 diabetes and normal ageing as subendocardial contractile function is impaired which results in less effective counteraction of the twisting motion by subendocardial myofibers, resulting in an increase in torsion (Lumens et al. 2006). This suggests there may be some pathological change causing loss of subendocardial contractile function relative to subepicardial function (Lumens et al. 2006). This impairment may be attributed to a subendocardial perfusion deficit, fibrosis or infarction (Lumens et al. 2006).

Pathogenesis

Advanced glycation end-products-Due to the involvement of many factors, the pathogenesis of diabetic cardiomyopathy has yet to be fully elucidated but one hypothesis which is becoming widespread is the impact of protein glycation on myocardial tissue. The combination of protein with glucose-derived carbonyls produces advanced glycation end products (AGE) which are increased in Type 2 diabetes (Bodiga et al. 2013). The sarcoplasmic reticulum which is the site of calcium (Ca^{2+}) release for myocardial contraction has a loss of function in Type 2 diabetes, leading to a decreased state of relaxation (Bouchard & Bose 1991). Mounting evidence is indicating a role of non-enzymatic glycation in altering proteins (namely SERCA) involved in Ca^{2+} cycling within the sarcoplasmic reticulum (Bodiga et al. 2013). In addition to this, AGE's have been found to increase cross linking of

proteins such as collagen, making them rigid and thereby reducing cardiac contractility (Bodiga et al. 2013).

Inflammation: A pro-inflammatory state exists in Type 2 diabetes. Cardiac function was strongly associated with inflammatory markers when assessed using MRI techniques and although the small sample size (N=13) was small and the cross sectional nature means causality cannot be inferred, it's likely that inflammation directly impacts the heart (Diamant et al. 2005). Reduction in cardiac inflammation in rodent models has led to beneficial effects (Bugger & Abel 2014).

Mitochondrial dysfunction and oxidative stress: Altered permeability of mitochondria, impaired mitochondrial respiratory capacity and increased oxidative stress have been observed in human diabetic hearts (Dhalla et al. 2014). Oxidative stress and the production of reactive oxygen species (ROS) damages proteins and phospholipids. Many studies have demonstrated increases in these damaging molecules, in both human and rodent hearts (Anderson et al. 2009; Boudina et al. 2007).

IR: Although insulin stimulates normal cardiac growth, IR leading to hyperinsulinemia has been linked to pathological hypertrophy, although this is not concrete. In mice it was shown that high levels on insulin led to chronic pressure overload in cardiac tissue due to mechanical stretch-induced activation of insulin pathways. This chronic pressure overload altered the ratio between cardiomyocyte size and vascularity, leading to hypoxia and cell death (Shimizu et al. 2010). This supports evidence that the use of insulin therapy to improve glycaemic control can increase risk of cardiovascular events (Gerstein et al. 2008).

Epigenetics: Type 2 diabetes is associated with changes in global gene expression and so there is a plausible link between altered myocardial micro-RNA content and changes in cardiac function. Dysregulation of micro-ribonucleic acid in mice was associated with diabetic cardiomyopathy (Feng et al. 2010).

Lipotoxicity: Under healthy phenotypes, most triacylglycerol is stored within adipocytes but accumulation in myocardial tissue is observed in Type 2 diabetes (Zhou et al. 2000). In the Zucker diabetic fatty rat, a 2-fold increase in myocardial triacylglycerol content accompanied changes in cardiac structure and function (Zhou et al. 2000). Ceramide which is a mediator of apoptosis, was 2-3 times higher than the control group (Zhou et al. 2000). It is clear that excessive deposits of lipids activate adverse signalling cascades, which can result in cell death, otherwise known as lipotoxicity, thereby affecting cardiac structure and function (McGavock 2006; Bugger & Abel 2014).

Despite many possible contributors leading to heart failure in Type 2 diabetes, there has been limited advance in detailing its time course and pathophysiology. Many of the theories above, have been nurtured through use of the Zucker diabetic rat which does not account for differences in the time course and remodelling processes between rodents and humans as well as the relative contribution of ischemia, autonomic neuropathy and hypertension in humans (Diamant 2012). Therefore the ability to generalise results to human cardiac tissue is limited.

Altered Cardiac metabolism

A feature of the diabetic heart is 'metabolic inflexibility' whereby an increase in NEFA uptake and decrease in glucose oxidation is demonstrated (Miki et al. 2013). Efficiency of ATP synthesis is reduced when NEFA is the dominant fuel as 23 oxygen (O_2) are required to oxidise palmitate whereas only 6 O_2 are required to oxidise one molecule of glucose. This metabolic inefficiency can be identified using the PCr/ATP ratio which has been shown to decrease by as much as 35% in Type 2 diabetes individuals displaying normal cardiac morphology (Scheuermann-Freestone et al. 2003). PCr/ATP measured non-invasively by P-MRS, is negatively correlated with plasma NEFA concentration (Scheuermann-Freestone et al. 2003) and is a strong predictor of total mortality, superior to ejection fraction (Neubauer et al. 1997).

It has been proposed that reduced glucose metabolism results from impaired glucose transport into myocardial cells. While it's true that hyperlipidaemia

and hyperglycaemia attenuate insulin stimulated glucose transport (Isfort et al. 2013), elevations in glucose flux enhance the mass action effect of glucose uptake and so it seems that problems in glucose metabolism occur downstream of the glucose transporters (Isfort et al. 2013). Various receptor signalling pathways have been identified as the cause of metabolic inflexibility in Type 2 diabetes and it's clear that the Randle cycle is manifest within cardiac tissue with high concentrations of NEFA inhibiting glucose oxidation (Randle et al. 1963). One example of this is the up regulation of Peroxisome proliferator-activated receptor α (PPAR α) seen in Type 2 diabetes (Finck et al. 2002). PPAR α is activated by intracellular NEFA which leads to stimulation of enzymes involved in lipid metabolism (Hafstad et al. 2009) and up regulation of pyruvate dehydrogenase lipoamide kinase isozyme 4, which suppresses glucose oxidation (Buchanan et al. 2005). Alterations in the activity of key enzymes have also been identified, such as phosphofructokinase and pyruvate dehydrogenase complex (Isfort et al. 2013). Mitochondrial dysfunction has also been linked to reduced metabolic efficiency. Increased activity of uncoupling proteins and increased O₂ cost due to NEFA oxidation, elevates mitochondrial ROS production in Type 2 diabetes (Boudina et al. 2007). This not only causes local damage but can lead to an increase in cytosolic ROS which has been linked to altered Ca²⁺ movement and ATP generation (Isfort et al. 2013).

A cause and effect relationship has yet to be established but alterations in cardiac metabolism frequently precede the development of ventricular dysfunction (Buchanan et al. 2005) which suggest that metabolic inflexibility contributes to cardiac dysfunction (Larsen & Aasum 2008). Another indicator that cardiac inflexibility and ventricular dysfunction are linked comes from pharmacological interventions whereby cardiac metabolism is normalised and cardiac function is improved (Aasum et al. 2008). That being said, Rijzewijk et al. (Rijzewijk et al. 2009) found no change in cardiac metabolism in Type 2 diabetes. The exact role cardiac energetics play in Type 2 diabetes heart disease is therefore still to be established.

Prevention and treatment

Little is known about appropriate prevention and treatment strategies to manage heart disease in Type 2 diabetes. Improved glucose control is beneficial for cardiovascular events yet not much is known about the effect on diabetic cardiomyopathy (Miki et al. 2013). Diastolic resting velocity (a measure of diastolic function) improved in Type 2 diabetes individuals controlling their blood glucose with insulin during a 3 week intervention so that changes in diastolic myocardial velocity at rest correlated with changes in fasting blood glucose (von Bibra et al. 2004). It is well documented that the use of metformin is beneficial for diabetic cardiomyopathy, with a lower incidence of mortality and heart failure in those taking the medication (Aguilar et al. 2011; Andersson et al. 2010). Whether the effects are due to a reduction in hyperglycaemia alone or other mechanisms, has yet to be established.

There have been limited studies looking at the effects of exercise on diabetic cardiomyopathy although the ones undertaken have illustrated favourable outcomes. Type 2 diabetes patients absent of cardiovascular disease underwent a 12 month exercise intervention which consisted of 150 mins of moderate exercise combining aerobic and resistance activity (Hordern et al. 2009). Post-hoc analysis revealed those who performed more vigorous activity significantly improved diastolic function and displayed an increase in myocardial strain rate (systolic deformation). 12 weeks of soccer training in Type 2 diabetic men, increased left ventricular end-diastolic diameter, two-dimensional strain and E/A ratio (Schmidt et al. 2013). Only one group has measured the impact of HIIT; 12 weeks led to increases improvements in diastolic and systolic function. All of these aforementioned studies used echocardiography which is less accurate than MRI (Grothues et al. 2002). The rest of the evidence comes from rodent models.

The above studies indicate exercise as a potential therapy but limited research in humans leaves a gap in knowledge of the most appropriate ways to effectively manage the burden of diabetic cardiomyopathy.

1.7 Summary of literature review

Section 1.3 introduced metabolic regulation as a finely tuned system in which major metabolic organs including the liver, muscles and adipose respond to hormones and environmental stimuli so fluctuations in energy balance throughout the day are met with appropriate action. Any disturbance can lead to metabolic disease such as NAFLD and Type 2 diabetes, which pose significant UK health burdens. Section 1.4 described the influence of lifestyle behaviours including sleep, physical activity, sedentary behaviour and exercise on metabolism. Section 1.5 provided an overview of normal cardiac structure, function and metabolism and how MRI can be used to measure these parameters. Finally, section 1.6 describes how those with Type 2 diabetes (a prominent metabolic disease) have altered metabolism, lifestyle behaviours and cardiac health. The term ‘cardio-metabolic disease’ arises from the fact that those with metabolic disease have an increased risk of cardiac complications. Lifestyle behaviours including sleep, physical activity, sedentary behaviour and exercise all influence cardio-metabolic health, yet physical inactivity and unhealthy lifestyles are characteristic of modern society. Therapies and strategies are urgently required to address the increase in unhealthy lifestyles and the rise in cardio-metabolic disease.

1.8 Aims of thesis

In light of this information, this thesis aims to answer the following questions:

- 1) Using a representative UK sample, can we better define the ‘unhealthy behavioural phenotype’ of those with cardio-metabolic disease, and therefore highlight which lifestyle behaviours remain significant unaddressed risk factors? (*Chapter 2*)
- 2) In those with metabolic disease but no overt cardiovascular disease, what (if any) are the pre-clinical cardiac changes observed, using sensitive MRI? (*Chapter 4*)
- 3) Can exercise be used as a therapy to improve cardiac health in those with metabolic disease? (*Chapter 5*)

Chapter 2 A cross sectional study of diet, physical activity, sedentary behaviour and sleep in 233,110 adults from the UK Biobank; *the behavioural phenotype of Cardiovascular disease and Type 2 diabetes.*

2.1 Introduction

CVD and Type 2 diabetes represent significant personal, economic and societal burdens. CVD accounts for a quarter of all UK deaths (British Heart Foundation 2015) and people with Type 2 diabetes carry twice the risk of developing CVD (Sarwar et al. 2010). With over 700 new cases of diabetes diagnosed daily (Diabetes UK 2015), total health care expenditure on diabetes is forecast to rise from 10% to 17% by 2035 (Hex et al. 2012). The inter-relationship between cardiovascular and metabolic disease is termed cardio-metabolic health, and reflects their common environmental and genetic antecedents. Those with both CVD *and* Type 2 diabetes have a particularly poor prognosis and require aggressive risk factor intervention (Association 1999).

Behavioural factors, spanning diet, physical activity, sedentary behaviour and sleep are major risk factors for the development of cardio-metabolic disease. The reduction in energy expenditure through 1) lack of physical activity and 2) increase in sedentary behaviours are risk factors for cardio-metabolic disease (Grontved & Hu 2011; Bell et al. 2014). Indeed, technological advancements of the 21st century have paved the way for sedentary behaviours such as watching television, driving and sitting at a computer becoming the 'norm' in modern society, so that physical inactivity is now the 4th leading cause of disease and disability in the UK (Murray et al. 2013). Both physical activity and television (TV) sitting are associated with cardio-metabolic health when viewed separately (Laaksonen et al. 2002; Grontved & Hu 2011) or together (Chu & Moy 2013; Petersen et al. 2014). It is becoming increasingly common to combine these movement behaviours, and results indicate stronger associations with metabolic health when viewed together (Bell et al. 2014).

An important lifestyle behaviour, but often forgotten, is sleep and this is strongly linked to cardio-metabolic disease (Liu et al. 2013; Shan et al. 2015). Sleep is vital for resetting homeostasis and regulating metabolism, yet changes in working patterns and increased demands on time means sleep debt is a growing issue.

Since the World Health Organisations global strategy on diet, physical activity and health (WHO 2004) there have been calls for countries to develop national policy approaches to these lifestyle behaviours (Bull et al. 2014). Indeed, in 2011 the UK government published physical activity recommendations (Department of health 2011b) and Eat Well was produced as a policy tool that defines government recommendations on healthy diets (Public Health England 2014b). Specific National Institute for Health and Care Excellence (NICE) recommendations for CVD and Type 2 diabetes recognise the importance of improving physical activity and diet, but guidance on sitting time or sleep behaviours has not been addressed (NICE 2012; NICE 2014). Nonetheless, knowledge of baseline behaviours in the population is lacking.

The UK Biobank is a large, population-based cohort study examining the interrelationships between environment, lifestyle and genes with the aim of improving prevention, diagnosis and treatment of a wide range of serious and life threatening diseases (UK Biobank 2007). Extensive baseline measures were taken in >500 000 UK adults and over the next few decades will allow us to better understand why some people develop particular diseases and others do not.

2.2 Study aims

- 1) to observe the differences in lifestyle behaviours simultaneously across cardio-metabolic disease
- 2) to explore clustering of unhealthy non-diet behaviours across disease groups

2.3 Methods

2.3.1 Population and Study Design

Around 9.2 million invitations were mailed to recruit 502,664 adults (response rate 5.5%) aged between 37-73 years. They aimed for this age group because they are the group who are at risk of developing a number of chronic diseases over the next few decades. Recruitment occurred between 2007-10, via 22 assessment centres located across the UK. The UK Biobank study was approved by the North West Multi-centre Research Ethics Committee, the England and Wales Patient Information Advisory Group, and the Scottish Community Health Index Advisory Group. Participant written informed consent was obtained prior to data collection. All data extracted by users was de-identified for analysis.

2.3.2 Assessment centre visit

During an assessment centre visit there were 5 stations; consent, touchscreen questionnaire, verbal interview, physical measures and blood/urine sample collections (Figure 27).

- 1) Participants were guided through the consent procedure online, with a member of staff present to answer any questions and verify consent before moving onto the next station.
- 2) The touchscreen questionnaire station lasted around 40 mins and covered questions on socio-demographics, occupation, lifestyle, early life exposures, cognitive function, family history of illness, and medical history.
- 3) A 5 minute interview was conducted in which disease diagnosis was entered and verified by a UK Biobank nurse, followed by 2 blood pressure measurements (one minute apart) using Omron 705 IT monitor connected directly to the computer.
- 4) A number of physical measures were taken, including weight using the Tanita BC-418MA body composition analyser and height using a Seca 202 height measure, performed by trained staff. Participants were required to

remove shoes and heavy outer clothing and BMI was calculated from: weight(kg)/height(m)².

5) The final stage involved a blood collection from the antecubital fossa followed by a urine sample provided by the participant.

Figure 27 Stages of assessment centre and estimated time

Visit Station	Assessments undertaken	Estimated time (mins)
Reception	<ul style="list-style-type: none"> welcome & registration consent 	10
Questionnaire	<ul style="list-style-type: none"> touch-screen questionnaire cognitive function tests 	40
Interview (& blood pressure)	<ul style="list-style-type: none"> interviewer questionnaire blood pressure measurement 	10
Physical measurements	<ul style="list-style-type: none"> height (both standing & sitting) hip & waist measurement bio-impedance measurement hand-grip strength heel bone ultrasound spirometry 	15
Sample collection (& exit)	<ul style="list-style-type: none"> blood sample collected urine sample sought consent & result summary printed travel expense claim provided 	15
TOTAL		90

2.3.3 Diet, sitting, physical activity and sleep: measurement and data processing

Diet

Diet intake was reported using the Food Frequency Questionnaire (Bain et al. 1985) in which a number of questions were used based around commonly eaten food groups (Table 3 + Appendix A). Information on fresh/dried fruit, salad and cooked/raw vegetables were used to control for diet in logistic regression (see statistical analysis below). A binary variable was created which identified individuals who did and did not meet the UK's current guidelines on fruit and vegetable consumption (5 portions per day) (Public Health England 2014c). 4 questions on fruit and vegetable consumption were asked:

-“On average how many heaped tablespoons of COOKED vegetables would you eat per DAY? (Do not include potatoes; put '0' if you do not eat any)”

-“On average how many heaped tablespoons of SALAD or RAW vegetables would you eat per DAY? (Include lettuce, tomato in sandwiches; put '0' if you do not eat any)”

-“About how many pieces of FRESH fruit would you eat per DAY? (Count one apple, one banana, 10 grapes etc as one piece; put '0' if you do not eat any)”

-“About how many pieces of DRIED fruit would you eat per DAY? (Count one prune, one dried apricot, 10 raisins as one piece; put '0' if you do not eat any)”

Cooked vegetables, salad/raw vegetables and dried fruit were all divided by 3, so that portion sizes were aligned to current recommendations. For example, the NHS portion sizes for cooked vegetable is around 3 heaped tablespoons, therefore the inputted value was divided by 3 to count as 1 portion.

Physical activity

Physical activity was assessed using 6 items in the validated Short International Physical Activity Questionnaire (IPAQ) (Craig et al. 2003) which covers the intensity and duration of walking, moderate and vigorous activity in the past 7 days (see Appendix A for example questionnaire). Data processing rules published by IPAQ were followed (IPAQ 2005) which included:

-Only values of 10+ mins were included, responses of less than 10 mins (and their associated days) were re-coded to zero.

-All walking, moderate and vigorous time exceeding 180 mins were truncated to 180 mins.

-All cases in which the sum of all walking, moderate and vigorous time was >960 mins were excluded from analysis.

-Those who reported >7 days for either walking, moderate, vigorous were excluded from analysis.

-For each category (walking, moderate, vigorous), if one of days or mins was zero and the other was missing, recode the missing value to zero.

-If both mins and days were missing for one category, but all other 4 questions covering the 2 other categories were complete, recode the missing values to zero,

-Finally, only individuals who had values for all 6 questions were included in analysis.

Time spent in vigorous, moderate, and walking activity was weighted by the energy expended for these categories of activity, to produce METs.min/week of each category. 'Total physical activity' was the sum of walking, moderate and vigorous METs.min/week (see equations below).

Walking METs.min/week = 3.3 * walking minutes * walking days

Moderate METs.min/week = 4.0 * moderate-intensity activity minutes * moderate days

Vigorous METs.min/week = 8.0 * vigorous-intensity activity minutes * vigorous-intensity days

Total physical activity METs.min/week = sum of Walking + Moderate + Vigorous METs.min/week

Sitting

TV time related sitting referred to as 'sitting time' (Wilmot et al. 2012) was used as a marker of sedentary behaviour. Participants were asked; "In a typical day, how many hours do you spend watching television?" based on previous literature (Hu et al. 2003). This was asked twice to those who responded >8 hours, therefore high values were deemed robust but truncated at 17 hours, to allow for 7 hours sleep (average sleep duration across groups)

Sleep

To measure sleep duration, participants were asked “*About how many hours sleep do you get in every 24 hours? (please include naps)*”. This was asked twice to those who responded >12 hours, therefore high values deemed robust but truncated at 14 hours.

2.3.4 Disease Categories

Health status was entered and verified by a UK Biobank nurse, during the verbal interview. Four disease groups were identified spanning cardio-metabolic health. 1) *Healthy reference group*: Participants with no disease listed were classified as the ‘no disease’ group. 2) *Cardiovascular Disease*: Based on the International Classification of Diseases-10 (WHO 2015a) and a clinician opinion, diseases to include in the ‘CVD’ group were selected and any patients with Type 2 diabetes were excluded from this group (a list of diseases included in the CVD group can be found in Appendix A). 3 and 4) *Type 2 diabetes*: participants who were entered as having ‘diabetes’ or ‘type 2 diabetes’ were selected. Those taking insulin within their first year and <35 years old were excluded to reduce the likelihood of Type 1 and monogenic forms of diabetes. Those *without* and *with* CVD were separated into ‘Type 2 diabetes without CVD’ and ‘Type 2 diabetes + CVD’, respectively (Figure 28).

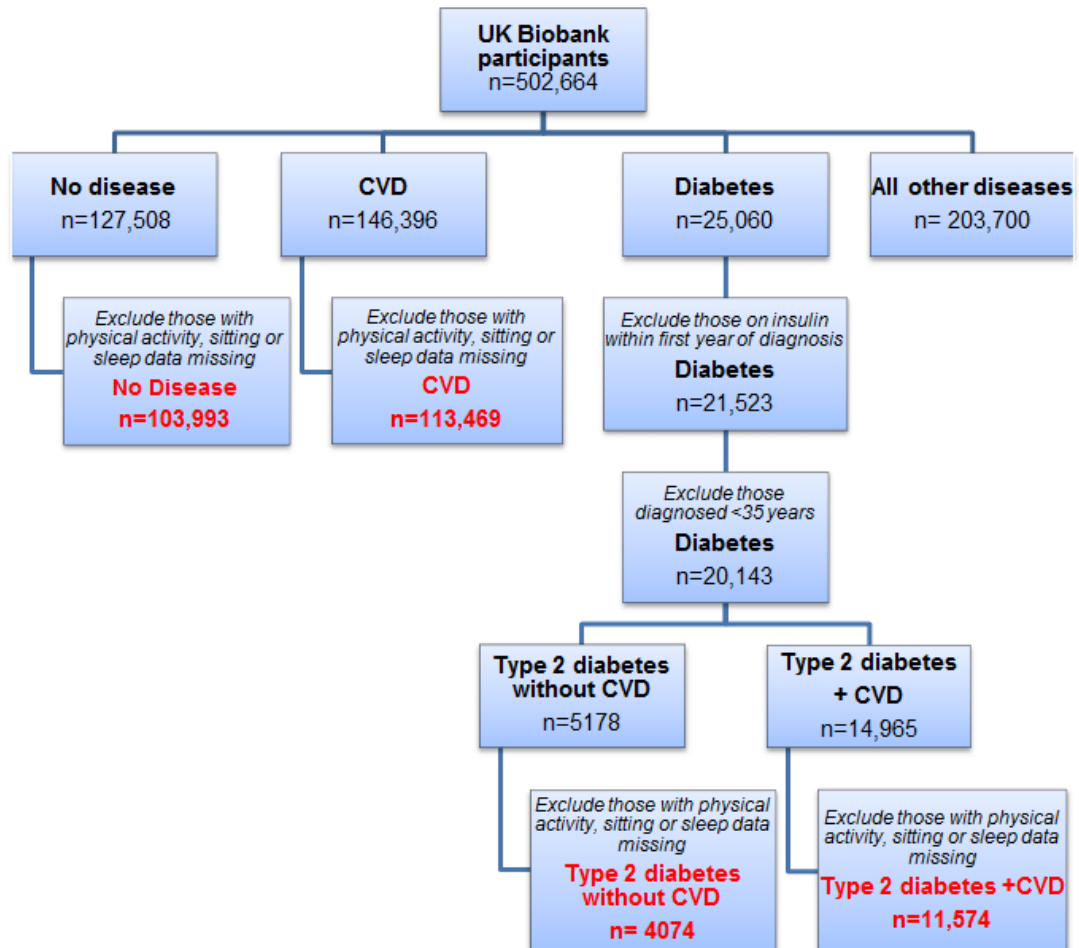
2.3.5 Statistical analysis

All Data analyses were performed using SPSS, version 21.0 (IBM, Armonk, NY, USA). Individuals with missing data on total physical activity, sitting time or sleep were excluded (Figure 28+ Appendix A) shows the socio-demographics of missing cases which were similar to the main cohort but had a lower % of males across all groups. Townsend deprivation Index was used as a measure of socio-economic status, by combining census data and post-codes of participants. The index combines information on housing, employment, car availability and social class, with higher values indicating greater deprivation. Townsend deprivation index was categorized into five groups based on the quintile demarcators for the ‘no disease’ group.

Total physical activity, vigorous, moderate and walking minutes alongside sitting time were categorised into 4 groups based on the quartile demarcators

for the 'no disease' group. Total physical activity groups were labelled as 'low physical activity' (lowest quartile: ≤ 918 METs.min/wk) and 'high physical activity' (highest quartile: $>3706-19,278$ METs.min/wk) and sitting time was labelled as 'low sitting time' (lowest quartile: ≤ 1 hour/day) and 'high sitting time' (highest quartile: >3 hour/day). As sleep duration shows a 'U' shaped relationship with diabetes risk (rather than a linear relationship like physical activity and sitting) the data was split using pre-defined thresholds from the literature. <7 hr, $7-8$ hr, >8 hr cut points were used based on a recent meta-analysis (Shan et al. 2015).

Figure 28 Flow chart demonstrating how disease groups were defined. Final 4 disease groups shown in red



Sleep duration was labelled as 'poor sleep' (<7 or >8 hours/night) and 'good sleep' (7-8 hours/night). Due to the large sample size, Pearson's chi squared deemed any small difference in group proportions as significant, therefore these results are not reported.

Non-diet lifestyle behaviours (including physical activity, sitting time and sleep duration) were further analysed across cardio-metabolic disease groups. Binary logistic regression was used to determine the odds of reporting low physical activity, high sitting time, and poor sleep separately, according to disease group. We also looked at the clustering of these behaviours. Participants were categorised as having an 'unhealthy phenotype' if they were categorised in all of the following groups; low total physical activity, high sitting time and poor sleep. Adjusted odds ratios, with 95% confidence intervals were reported. All logistic regression models were adjusted for: age (reference="40-49"); gender (reference="Female"); BMI (reference="<18.5-24.9"); Townsend Deprivation Index (reference="least deprived"); Ethnicity (reference="White/British"); Alcohol (reference="never"); Smoking (reference="Never"); Meets fruit/veg guidelines (reference="YES"). Of the 233,110 cohort, data was missing for; BMI (0.006%), Townsend Deprivation Index (0.002%), Ethnicity (0.003%), smoking status (0.003%), alcohol status (0.001%), and fruit and vegetable guidelines (0.015%) therefore these cases were excluded from the logistic regression models. All statistical tests were two-sided and significance was set at $p < 0.05$.

2.4 Results

Of the 502,664 UK Biobank participants, after excluding those with missing data or who were likely to have Type 1 diabetes, there were 103,993 (21%) with 'no disease', 113,469 (23%) with 'CVD', 4074 (1%) with 'Type 2 diabetes without CVD', and 11,574 (2%) with 'Type 2 diabetes + CVD' (Figure 28). As expected with cardio-metabolic disease progression, the proportion of males and those aged >60 years old increased, as did those classified as obese (Table 2). There was a marked increase in obesity, with numbers almost quadrupling in the 'Type 2 diabetes + CVD' group, compared to disease free individuals (60.0% vs. 15.0%). The 'No disease' group had a higher

proportion of White/British and least deprived individuals compared to cardio-metabolic diseases. According to the Townsend deprivation index, socio-economic status decreased across cardio-metabolic disease groups (Table 2).

Compared to the 'No disease' group, the 'Type 2 diabetes + CVD' group reported higher levels of previous smoking (n=5571 (48.3%) vs. n=30,960 (29.8%)) and alcohol (n= 853 (7.4%) vs. n= 2020 (1.9%)), but lower current alcohol consumption (n= 9891 (85.5%) vs. n= 98,354 (94.6%)). Dietary data indicate that three quarters of those with Type 2 diabetes have altered their diet within the past 5 years ('Type 2 diabetes without CVD': 75.5% *and* 'Type 2 diabetes + CVD': 75.3%) and also half never eat sugar ('Type 2 diabetes without CVD': 49.8% *and* 'Type 2 diabetes + CVD': 51.2%), which is proportionally more than the 'CVD' and 'No disease' groups. Around a third of the 'No disease' group met the UK's fruit and vegetable guidelines (29.8%) with an increasing trend across cardio-metabolic disease (Table 3). All other dietary behaviours are reported in Appendix A.

Table 2 **Socio-demographics of disease groups (n=233,110)**

	% within each disease group			
	No Disease (n=103,993)	CVD (n=113,469)	Type 2 diabetes without CVD (n=4074)	Type 2 diabetes + CVD (n=11,574)
SOCIO-DEMOGRAPHICS				
% Male	47.0	53.3	63.6	68.0
Age (n)	103,993	113,469	4074	11,574
37-49	35.6	12.5	13.5	6.0
50-59	35.9	30.2	32.6	27.6
60-73	28.6	57.4	53.9	66.4
BMI (n)	103,443	112,852	4048	11,478
<18.5-24.9 (under and acceptable weight)	42.9	22.0	14.9	7.2
25-29.9 (overweight)	42.1	44.4	40.5	32.8
≥30 (obese)	15.0	33.6	44.6	60.0
Townsend deprivation quintile (%)	103,861	113,323	4070	11,557
1 (least deprived)	21.9	19.7	17.7	14.7
2	20.8	19.9	17.3	17.4
3	20.7	19.9	18.9	18.5
4	19.6	20.0	20.8	21.0
5 (most deprived)	17.1	20.5	25.1	28.5
Ethnicity (n)	103,687	113,130	4060	11,528
White/British	94.6	95.0	85.6	89.9
Mixed	0.6	0.5	0.6	0.6
Asian	1.8	1.7	8.1	5.1
Black African	1.5	1.8	3.3	2.9
Chinese	0.5	0.2	0.5	0.2
Other	0.9	0.7	2.0	1.3

Table 3 Lifestyle characteristics of disease groups (n=233,110)

	% within each disease group			
	No Disease (n=103,993)	CVD (n=113,469)	Type 2 diabetes without CVD (n=4074)	Type 2 diabetes + CVD (n=11,574)
DIET				
Dietary change in past 5 yrs	103,902	113,300	4070	11,555
YES	28.9	45.9	75.5	75.3
Meets fruit/veg guidelines	102,798	111,554	3995	11,347
YES	29.8	32	35.7	36.6
“Never eat”	103,848	113,190	4039	11,527
Never eat sugar or foods/drinks containing sugar	14.8	21.0	49.8	51.2
PHYSICAL ACTIVITY				
Total Physical activity^a (METs.mins/wk)	103,993	113,469	4074	11,574
≤918 (Low physical activity)	25.0	30.5	35.4	40.1
>918-1902	25.0	24.2	22.5	22.2
>1902-3706	25.0	22.2	20.7	19.7
>3706-19278 (High physical activity)	25.0	23.2	21.3	18.0
Walking^a (mins/day)	103,993	113,469	4074	11,574
0-20	31.5	33.9	36.6	40.4
21-30	20.8	20.4	21.0	19.6
31-60	26.7	25.8	23.0	23.6
61-180	21.1	19.9	19.4	16.3
Moderate activity^a (mins/day)	103,993	113,469	4074	11,574
0-15	27.8	31.4	36.1	39.6
16-30	28.0	24.7	25.3	23.2
31-60	25.2	23.2	19.9	20.1
61-180	19.0	20.6	18.7	17.1
Vigorous activity^a (mins/day)	103,993	113,469	4074	11,574
0	34.2	46.1	49.7	56.5
1-20	20.3	19.6	19.7	18.0
21-45	21.7	16.7	14.5	13.1
46-180	23.7	17.6	16.1	12.3

Meets UK government physical activity guidelines ^b	103,993	113,469	4074	11,574
NO	42.6	47.8	52.2	56.1
SITTING				
Sitting time [TV]^a (hours/day)	103,993	113,469	4074	11,574
≤1 (Low sitting time)	26.6	16.2	15.4	10.5
>1-2	30.5	24.5	22.3	19.3
>2-3	22.6	24.5	24.1	22.8
>3 (High sitting time)	20.3	34.8	38.1	47.3
SLEEP				
Sleep duration ^c (hours/night)	103,993	113,469	4074	11,574
<7 (Poor sleep)	21.3	26.4	27.0	27.5
7-8 (Good sleep)	73.4	64.6	62.1	58.6
>8 (Poor sleep)	5.3	9.1	10.8	13.9

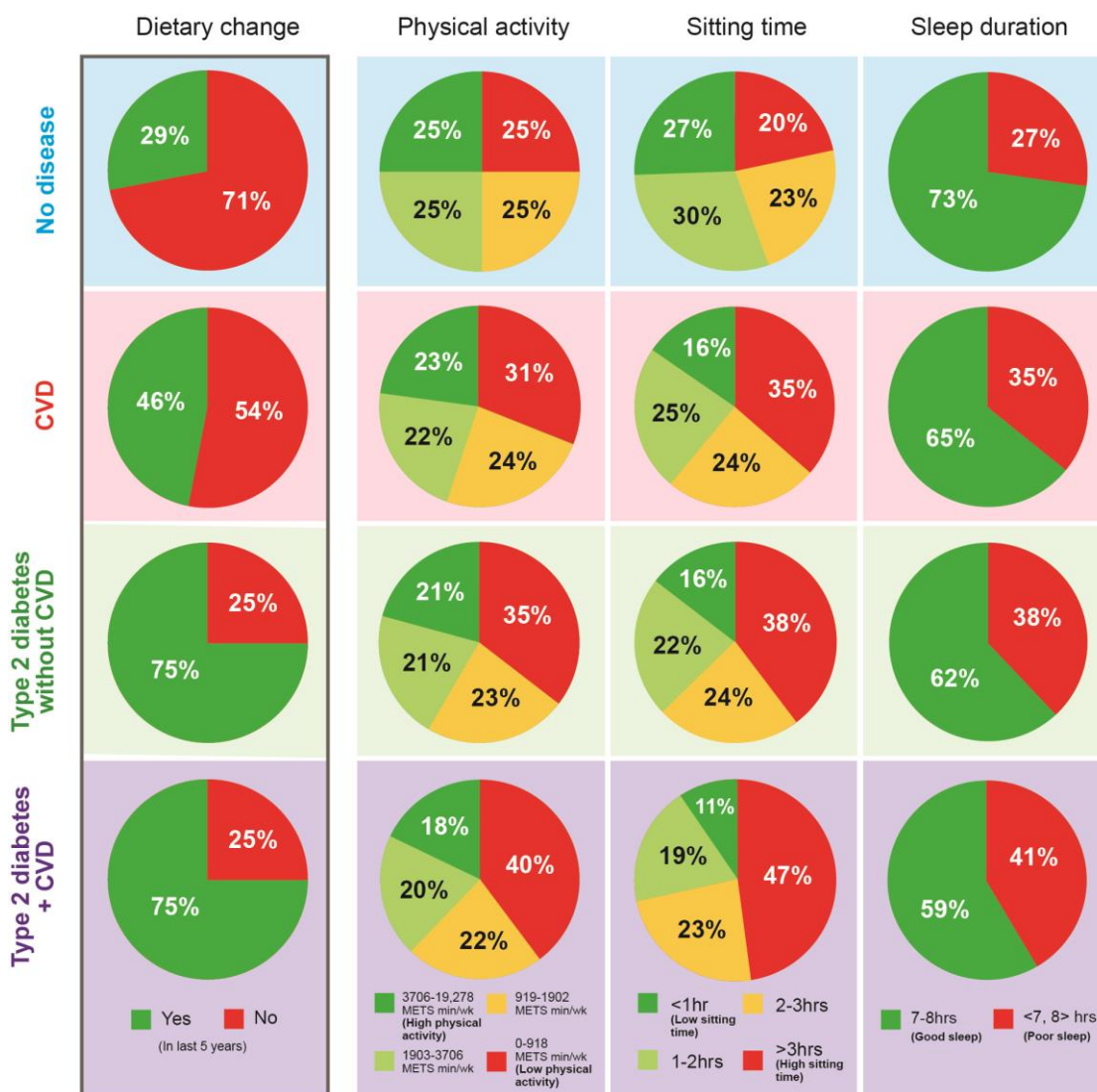
^a For total physical activity and sitting categories, quartiles were calculated from the 'No Disease' group so that their demarcators could be applied to disease group.

^b UK Government recommendations of 150mins of moderate or 75mins of vigorous activity per week

^c Physiological thresholds used rather than quartiles because the shape of the risk relationship is a U shape (not linear like Physical activity and TV sitting)

Total physical activity levels declined across cardio-metabolic disease groups (Table 3) + (Figure 29). Vigorous activity was the main contributor to the reduction in total physical activity levels, with a strikingly smaller proportion of adults in the 'Type 2 diabetes + CVD' group reaching the upper quartile of vigorous activity compared to the 'No disease' group (12.3% vs. 23.7%) (Table 3). The proportion of adults who reported high sitting time more than doubled in the 'Type 2 diabetes + CVD' group compared to the 'No disease' group (47.3% vs. 20.3%) (Table 3).+ (Figure 29). These results indicate that almost half of adults diagnosed with 'Type 2 diabetes + CVD' sit for >3 hours per day watching television. Almost three-quarters of the 'No Disease' group report optimal sleep but this proportion declined across cardio-metabolic disease. The proportion of poor sleepers (<7hrs and >8hrs) was higher in cardio-metabolic disease groups compared to the 'No Disease' group (Table 3) + (Figure 29).

Figure 29 Distribution of dietary change, physical activity, sitting time and sleep duration in people with No disease, CVD, Type 2 diabetes without CVD, or Type 2 diabetes + CVD. Red indicates unhealthy and green indicates healthy lifestyle behaviours. Non-diet lifestyle behaviours are separated from diet as they demonstrate worsening of behaviours with cardio-metabolic disease, which is absent with diet



Those with the most serious cardio-metabolic disease profile (Type 2 diabetes + CVD) were 70% (Odds [95% CI]: 1.71 [1.64 to 1.78]), 90% (1.92 [1.85 to 1.99]), and 50% (OR 1.52, 95%CI 1.46 to 1.58) more likely to report low physical activity, high sitting time and poor sleep respectively, compared to the 'No Disease' group (Table 4). The Odds of reporting all three unhealthy behaviours together was higher than reporting one of these lifestyle behaviours individually. Indeed, those in the 'Type 2 diabetes + CVD' group were three times more likely to report an 'unhealthy phenotype', (i.e. low physical activity, high sitting time and poor sleep) (3.29 [3.02 to 3.58]) even when controlling for age, gender, BMI, Townsend Deprivation Index, Ethnicity,

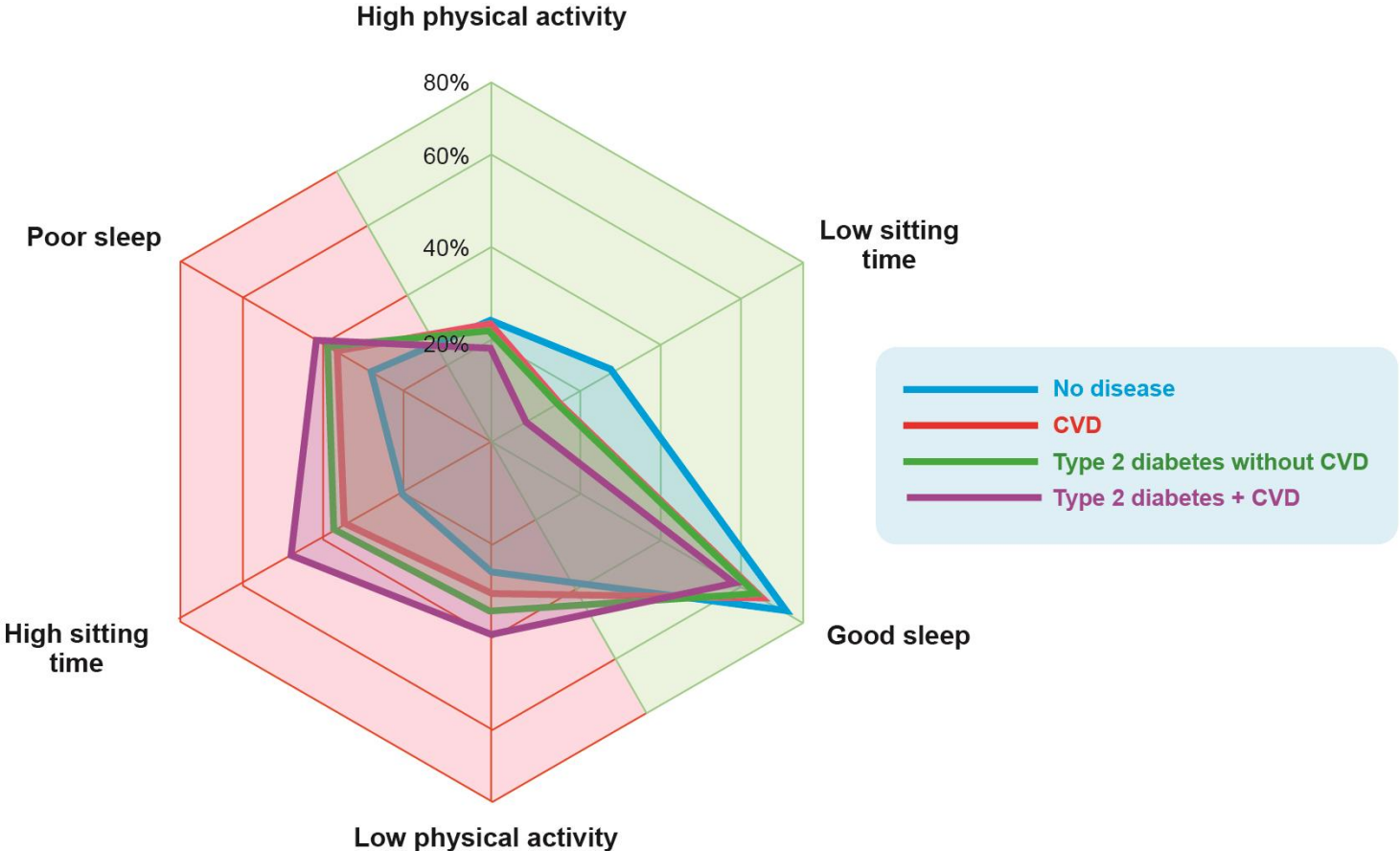
Alcohol, Smoking, and meeting fruit/veg guidelines (Table 4). BMI and cardio-metabolic disease are strongly linked, yet the odds of being obese in the 'Type 2 diabetes + CVD' group were less (2.77 [2.60 to 2.96]) than the odds of reporting an 'unhealthy phenotype'. The shift in unhealthy behaviours is visualised in Figure 30 which shows the movement from healthy behaviours (green / right) to unhealthy behaviours (red / left).

Table 4 Odds (95% CI) of reporting low physical activity, high sitting time, poor sleep and all behaviours combined across cardio-metabolic disease. *All models adjusted for age, gender, BMI, socio-demographic (Townsend deprivation and ethnicity), smoking, alcohol and diet.*

	Low physical activity	High sitting time [TV viewing]	Poor sleep	Low Physical Activity + High Sitting + Poor Sleep
No Disease	1.00	1.00	1.00	1.00
CVD	1.23 [1.20 to 1.25]	1.42 [1.39 to 1.45]	1.37 [1.34 to 1.39]	2.15 [2.03 to 2.28]
Type 2 diabetes without CVD	1.43 [1.34 to 1.53]	1.59 [1.49 to 1.69]	1.38 [1.30 to 1.47]	2.14 [1.85 to 2.48]
Type 2 diabetes + CVD	1.71 [1.64 to 1.78]	1.92 [1.85 to 1.99]	1.52 [1.46 to 1.58]	3.29 [3.02 to 3.58]

Figure 30

Radar chart showing the proportion of adults in each group who were categorized as either 'high' or 'low' for total physical activity or sitting time, or 'good' or 'poor' for sleep duration. Green side indicates healthy non-diet lifestyle behaviours whereas red side indicates unhealthy non-diet behaviours. There is a shift towards unhealthy behaviours with cardio-metabolic disease.



2.5 Discussion

This is the largest cohort study to simultaneously assess diet, physical activity, sedentary behaviour and sleep across cardio-metabolic disease groups and non-disease group. The results indicate that compared to disease free individuals; 1) those with cardio-metabolic disease report less physical activity, higher sitting and poorer sleep patterns, 2) non-diet unhealthy lifestyle behaviours were clustered in people with 'Type 2 diabetes + CVD'; they were three times more likely to report low physical activity, high sitting *and* poor sleep at the same time, and 3) people with cardio-metabolic disease had changed their diet and were less likely to consume sugary foods. These results suggest that recommendations to change diet are reaching those with cardio-metabolic disease; yet, low physical activity, high sitting and poor sleep are significant unaddressed cardio-metabolic risk factors.

2.5.1 Diet

People with Type 2 diabetes reported an increased likelihood to have changed their diet in the past 5 years and less likely to eat sugary foods compared to people with CVD or disease free individuals. Dietary change is aligned to the current treatment advice for people with Type 2 diabetes (NICE 2015) and suggests that patients are acting upon, or at least aware of, dietary advice. The food frequency questionnaire did not allow us to measure energy intake; therefore, it is possible that, although participants had changed their diet, they ate more. To address excess calorie intake we have controlled for BMI as a means to manage excess calorie intake. In contrast to those with Type 2 diabetes, people with CVD consume more sugary foods than people with diabetes and are less likely to have changed their diet, as advised by current advice (NICE 2014). Cardio-protective diets are promoted to reduce cholesterol and blood pressure in CVD (Kris-Etherton 2002; de Lorgeril et al. 1999). Dietary advice remains the pillar of national guidelines for the management of Type 2 diabetes, with evidence reporting that dietary changes can significantly improve glycaemic control (Brand-Miller et al. 2006) and even reverse Type 2 diabetes (Lim et al. 2011; Andrews et al. 2011). Analysis of the self-report diet behaviour from the UK Biobank cohort

suggests that national dietary messaging is reaching those with Type 2 diabetes, but not those with CVD.

2.5.2 Physical activity

Those with cardio-metabolic disease report less physical activity than healthy counterparts, with vigorous activity being the largest contributor to this difference. A recent prospective meta-analysis confirmed a dose-response relationship between physical activity and Type 2 diabetes, with the strongest associations seen with vigorous activity (Aune et al. 2015). Physiologically there are a number of cardio-metabolic benefits with activity, including; improvements in lipid oxidation (Trenell et al. 2008), reductions in adiposity (Mozaffarian et al. 2011), and improved peripheral glucose uptake (Hayashi et al. 1997). Acute vigorous activity stimulates greater peripheral adaptations compared to non-vigorous activity (Mayer-Davis 1998), which may explain the stronger association with vigorous activity and cardio-metabolic disease. The government produced physical activity guidelines which encourage individuals to perform at least 150 mins of moderate activity or 75 mins of vigorous activity weekly (Department of health 2011b). Based on these recommendations, 43% of the 'no disease' group *do not* perform adequate physical activity levels and the percentage rises with cardio-metabolic disease.

2.5.3 Sitting

Sedentary behaviour was higher in those with cardio-metabolic disease compared to healthy adults. The first longitudinal study to look at sitting and cardio-metabolic risk found that change in sitting duration over 5 years was associated with waist circumference and clustered cardio-metabolic risk score, independent of physical activity (Wijndaele et al. 2010). Subsequent meta-analyses have revealed a dose response relationship between sitting and Type 2 diabetes or CVD, the highest levels of sitting leads to the highest risk of disease (Grontved & Hu 2011). More than 3 hours of daily sitting was strongly linked to all-cause mortality, (RR [95% CI] 1.30 [1.06-1.56]) (Grontved & Hu 2011) suggesting that those in the highest quartile of sitting in the UK Biobank cohort are exposing themselves to detrimental health consequences. Sedentary behaviour is characterised by low muscle activity,

which has a direct physiological health impact. Skeletal muscle is the largest insulin sensitive organ in the body, accounting for 80% of insulin stimulated glucose disposal, and reduced muscular contraction with high sitting time leads to insulin resistance and hyperglycaemia (Hamburg et al. 2007).

Sedentary behaviour also leads to reduced lipoprotein lipase activity which is linked to lower plasma HDL cholesterol and raised triacylglycerol content (Hamilton et al. 2007) raising cardio-metabolic risk. Results from the UK Biobank may reflect reverse causality whereby individuals with cardio-metabolic disease are sick and therefore more likely to have high sitting times. That being said, the aforementioned research has identified negative health outcomes associated with too much sitting. NICE guidelines for Type 2 diabetes briefly mention that sitting time should be reduced, but no specific guidelines are made (NICE 2012).

2.5.4 Sleep

These data reveal that as cardio-metabolic disease worsens, the proportion of individuals reporting short or long sleep increases. We have defined optimal sleep as 7-8 hours based on a recent review (Shan et al. 2015) and our findings support previous observational studies which show a 'U shaped' relationship between sleep duration and cardio-metabolic disease.

Physiological studies show sleep plays an integral role in metabolic regulation (Trenell et al. 2007) with sleep restriction inducing insulin resistance and loss of circadian hormone changes. Indeed, the acute effects of sleep shortening are powerful. Sleep restricting healthy young men from 8 hours/night to only 4 hours/night for one week, induces insulin resistance to a similar extent to people with Type 2 diabetes (Spiegel et al. 1999). Sleep shortening also effects hormones that control appetite (Taheri et al. 2004), elevating ghrelin and reducing leptin, which could explain the strong link between sleep deprivation, raised energy intake and weight gain (Taheri et al. 2004). Impaired sleep and cardiovascular disease are similarly associated, (Liu et al. 2013) mediated through weight gain and inflammation (Miller & Cappuccio 2007). Persistent long sleep and increases in sleep duration over a 5 year period have been linked to higher Type 2 diabetes incidence (Ferrie et al. 2015) however, the physiological impact of long sleep is yet to be fully

understood. Although there will be an impact of long sleep on the opportunity to be physically active during wakefulness, more work is needed to explore the impact of normalising sleep in people who have long sleep. The results from this study are observational and cannot infer causality, however, previous studies have demonstrated the potent effects of sleep on physiological function, highlighting poor sleep as a potential therapeutic target in people with CVD and/or Type 2 diabetes.

2.5.5 Clustered Lifestyle Behaviours

The results from this large population based study, also indicate an inverse relationship between an 'unhealthy behavioural phenotype' encompassing low physical activity, high sitting time and poor sleep, with cardio-metabolic disease. The 'Type 2 diabetes + CVD' group, who have a particularly poor prognosis, were more likely to report low physical activity, high sitting time *and* poor sleep compared to all other groups. In the context of cardio-metabolic disease and obesity, it is becoming increasingly common to combine physical activity and sitting as a joint association (Chu & Moy 2013; Petersen et al. 2014). We have added sleep into our analysis as we propose all three behaviours are interdependent in their influence upon metabolic control. Indeed, the clustering of these behaviours produces higher odds with cardio-metabolic disease compared to individual behaviours. All three behaviours influence metabolic control. During sleep, there is a reduction in glucose utilisation, with an overall rise in plasma glucose (Van Cauter et al. 1997). In contrast, throughout waking hours, physical activity stimulates peripheral glucose uptake (Hayashi et al. 1997) and is important for maintenance of euglycaemia. Physical activity may be viewed as an activator of metabolism whereas sleep is vital for restoring and re-setting homeostasis, largely through energy regulation and repair.

The concomitant nature of all 3 behaviours means the imbalance between sitting, physical activity and sleep has potentially large cardio-metabolic consequences. Sleep restriction reduces β -cell insulin release (Spiegel et al. 1999), alongside impairing phosphorylation of AKT, a crucial step in the insulin signalling cascade, which reduces insulin sensitivity (Broussard et al. 2015). These factors, alongside the reduction in peripheral glucose uptake

with high sitting and low physical activity, creates an insulin resistant state, the prominent feature underlying cardio-metabolic disease (Ferrannini et al. 1991). There is also evidence that sleep directly influences physical activity, with short term sleep deprivation significantly reducing habitual physical activity as well as shifting the intensity towards lower levels (Schmid et al. 2009). The present data suggest that people with CVD and / or Type 2 diabetes are more likely to be exposed to a potent negative 'behavioural phenotype' consisting of low physical activity, high sedentary behaviour and poor sleep simultaneously, but whether this is a cause or consequence of cardio-metabolic disease cannot be elucidated from this study.

2.5.6 Implications for care teams, policy makers and people with cardio-metabolic disease

Data from the UK Biobank suggest that poor non-diet lifestyle behaviours are prominent behaviours in cardio-metabolic disease. Our findings should not be taken to understate the importance of diet in cardio-metabolic health. A balanced diet and weight management are critical and efforts should continue to support people accordingly. However, the government recently described physical activity as a 'key health priority in it's own right' (Department of Health 2015) highlighting the importance of strategic planning with various sectors spanning transport, infrastructure and training of health care professionals. In 2014, Public Health England produced a framework to embed physical activity into the fabric of daily life (Public Health England 2014a) and the first national NHS prevention programme designed to prevent Type 2 diabetes through diet and physical activity interventions (Public Health England 2015). The present data reinforces the need for evidence based and effective programmes for physical activity for people with CVD and Type 2 diabetes.

Awareness of the importance of sedentary behaviours in chronic disease lags physical activity, but is growing rapidly. In 2010, the department of health and the sedentary behaviour and expert working group recommended that more emphasis needed to be placed on minimising time spent sedentary (Biddle et al. 2010). Indeed, NICE guidelines for Type 2 diabetes prevention note the importance of reducing sitting time (NICE 2012), yet guidelines and

techniques for implementation are lacking. In contrast to physical activity and sitting, NICE guidelines for CVD and Type 2 diabetes do not comment on sleep, despite the present data revealing that one in three of people with CVD and nearly half of people with Type 2 diabetes sleep either too much or too little.

A major finding from the present data was the clustering of physical activity, sitting and sleep behaviour. This is important as, to date, intervention studies have focused on changing a single lifestyle behaviour, with very few targeting multiple lifestyle behaviours (King et al. 2015). Given the clustering of these non-diet lifestyle behaviours, exploration of interventions incorporating physical activity, sitting *and* sleep together may add value and should be the focus of future policies and programmes.

2.5.7 Strengths and limitations

This data holds strength in the large sample size and detailed measurements. The population-based design allows simultaneous presentation of behaviours in people with different stages of cardio-metabolic disease, controlling for key factors including age, sex, socioeconomic status and BMI. However, the study is not without limitation. The response rate was only 5.5% which means the data is unlikely to be a true representative sample of the UK. The cross sectional nature means we cannot infer whether these unhealthy lifestyle behaviours precede or were preceded by cardio-metabolic disease. Over time, as the UK Biobank cohort progresses, it is hoped that observations about causality will be added. Lifestyle behaviours were self reported and not objectively measured. However, all questionnaires are validated and self-report allows these measures to be applied to large numbers of people. As such, the approach provides a powerful macro view of behaviours. Using TV viewing time is a narrow measure of sedentary behaviour and does not take into account breaks in sedentary time, which are important in metabolic control (Healy et al. 2008). Nonetheless, TV-viewing time is the most commonly used measure of sedentary behaviour in epidemiological studies and has good test-retest reliability (Clark et al. 2009). Calculation of total daily sitting time, which is a more complete measure, was not possible from the UK Biobank questionnaire used. With these limitations

noted, the strengths of the present data mean that it has both scientific and practical implications.

2.6 Conclusions

In summary, the present data demonstrates that those with more advanced cardio-metabolic disease undertake too little physical activity, sit too much and have poor sleep yet report important positive dietary changes within the past 5 years. These non-diet lifestyle behaviours are clustered, and indeed those with the worst cardio-metabolic disease are three times more likely to display an 'unhealthy behavioural phenotype' compared to disease free individuals, independent of age, gender, BMI and socioeconomic status. These novel data highlight that there is a specific behavioural phenotype of cardiovascular disease and Type 2 diabetes that may place these people at excess risk of worsening cardio-metabolic health. Strategies are required to address physical activity, sedentary behaviour and sleep to assist patients, care teams and policy makers in making effective decisions for the management and prevention of cardiovascular disease and Type 2 diabetes.

The next chapter will describe the methods used for the cardio-metabolic patient studies that were undertaken and form the remainder of this thesis.

Chapter 3 Methodology for patient studies

3.1 Recruitment strategy

Patients with Type 2 diabetes were recruited from advertisements in local newspapers, council newsletter, hospital notice boards, and community support groups. The inclusion criteria were as follows:

- Diagnosed with Type 2 diabetes and controlled by diet and/or metformin only.
- Not taking part in regular exercise (≥ 60 mins moderate-vigorous activity per week)
- Not undergoing dietary or medication change
- Not taking insulin/Sulfonylurea/ Thiazolidinediones/Beta-blockers
- Aged 30-70
- No contraindications to exercise testing according to the American college of sports medicine (ACSM et al. 2010).
- Can give informed consent
- No contraindications for MRI scanning (e.g. pacemaker, aneurysm clip, complete MRI checklist)
- No heart or kidney disease

3.2 Informed consent process

Patients received a study information sheet to read at least 24 hours before written informed consent was obtained. On the day of consent, individuals attended the clinical research facility without being fasted to maximise alertness. The study aims, procedures and time commitments were emphasised before patients were given space to ask any questions regarding the study. If patients were happy to proceed, a consent form was signed and dated (Appendix B) by both the patient and research investigator in accordance with local ethics guidelines. It was stressed that patients were free to withdraw at any point during the study and that GP's would be made aware of their participation. All studies were approved by the Newcastle and North Tyneside Research Ethics Committee.

3.3 Screening visit

Prior to any experimental procedures, patients attended a screening visit to assess their health status and check they met the study inclusion criteria. The visit included a physical examination and cardiopulmonary exercise test.

3.3.1 Physical examination

The Physical Activity Readiness Questionnaire (PARQ) was completed and a full medical history (Appendix C) was conducted prior to the physical examination which included auscultation of heart and lungs, lower extremity examination for oedema, inspection of the skin (specifically lower extremity in diabetes due to peripheral neuropathy), reflex function, and abdomen evaluation (Appendix C).

Weight and height were determined using an electronic scale and stadiometer (SECA, Birmingham, UK), respectively, with patients shoeless but wearing clothes. Waist circumference was measured as a horizontal measure taken at the narrowest part of the torso and hip circumference was measured at the maximal circumference of buttocks, in accordance with the American College of Sports Medicine guidelines (ACSM et al. 2010). BMI was calculated as $\text{body weight(kg)/height}^2(\text{m})$.

After 5 minutes of rest, patients received a 12 lead electrocardiogram (ECG) (Mac 500, Germany) in a supine position followed by a blood pressure measurement (Welch Allyn Adult 11) in a seated position

Patients with any contraindications to exercise testing (Trenell 2009) were excluded from the study and unable to proceed with the exercise test. The documents in Appendix C were used to determine if patients had any contraindications for exercise testing

3.3.2 Cardiopulmonary exercise test

A progressive exercise test was performed using an electronically braked semi-recumbent cycle ergometer (Corival Lode BV, Groningen, The Netherlands) to determine $\text{VO}_{2\text{peak}}$ and safety of exercise. After a 5 min warm up at 25W, resistance was increased by 1W every 6 seconds until participants reached volitional exhaustion, could no longer maintain 60

revolutions per minute (RPM), or could not continue due to contraindications (Appendix B) (Trenell 2009) .

Expired gases were collected using a Hans Rudolf breathing mask and analysed online for ventilation, oxygen consumption (VO_2) and carbon dioxide elimination (VCO_2) (Cortex metalyser 3B, Leipzig, Germany). A test-retest correlation demonstrated very good reliability for VO_2 (0.969), VCO_2 (0.953), and Ventilation (0.953) using the MetaLyzer 3B (Meyer et al. 2001). The calorimeter gas analysers were calibrated before every measurement for gas, volume, and ambient air pressure. Lactate threshold, RER, $\text{VO}_{2\text{peak}}$ and maximum workload (W) could be obtained from expired gases. Blood pressure was measured every 3 mins (Suntech Tango+, Suntech Medical Ltd, Oxford, UK) and continuous heart rhythm via a 12-lead ECG (Custo med GmbH, Otto- brunn, Germany).

Participants were required to abstain from eating for a minimum of 2 hours prior to the commencement of each test, and from vigorous exercise 24 hours prior to the test. Participants were also instructed to not consume alcohol and caffeine containing foods and beverages on test days.

3.4 Magnetic Resonance imaging

A 3.0 Tesla Philips Intera Achieva scanner (Best, NL) was used for all MRI examinations. Prior to any examination, a questionnaire was completed by patients twice to check they were eligible for MRI scans (Appendix D).

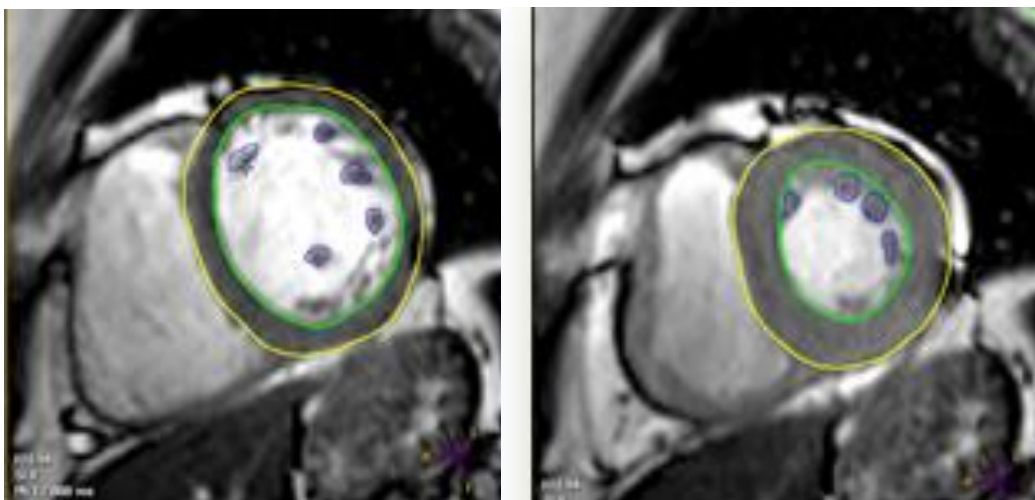
3.4.1 Cardiac cine imaging

A dedicated six-channel cardiac coil (Philips) was used with the participants in a supine position and ECG gating. Short-axis balanced steady-state free precession images were acquired covering the left ventricle (FOV = 350 mm, TR/TE = 3.7/1.9 ms, acceleration factor 17, flip angle 40° , slice thickness 8 mm, 0mm gap, 14 slices, 25 phases, resolution 1.37 mm).

Cine magnetic resonance imaging analysis was performed using a Viewforum workstation (Philips, NL). The short axis slices at end-diastole and end-systole were used to manually trace endocardial and epicardial borders

with papillary muscles excluded from volume calculations but included in calculations of left ventricular mass (Figure 31).

Figure 31 Short axis slice of the left ventricle at (left) end-diastole and (right) end-systole. Green trace shows the endocardial border, yellow trace the epicardial border and blue trace the papillary muscles.



The apical slice was defined as the last slice showing inter cavity blood pools and the basal slice where at least 50% of the blood volume was surrounded by myocardium (Hudsmith et al. 2005). The inter-ventricular septum was included as part of the left ventricle. Left ventricular mass, ejection fraction, end-systolic and end-diastolic volumes were calculated. Myocardial mass was determined by multiplying the tissue volume by 1.05 g/cm^3 , the specific density of myocardium. The body surface area was estimated from the subjects' weight and height according to the formula of Dubois and Dubois (Du Bois & Du Bois 1989) and this was used to standardise the measurements for subject size (denoted by the suffix "index").

To examine possible diastolic dysfunction, blood pool volumes were calculated across all phases to look for the characteristic two phase expansion of the blood pool. The left ventricular volume measurements (25 per cardiac cycle) were plotted against time. The data were then smoothed using a piecewise cubic spline algorithm and oversampled into 256 data points to create a volume-versus-time-curve. The rate of change of blood pool volume was determined by taking the first derivative of this curve over the entire cardiac cycle. End-systole and end-diastole were defined as the

times of lowest and greatest volumes, respectively. The time point halfway between end-systole and end-diastole was defined as the diastolic midpoint. Four indexes of left ventricular diastolic function were determined from the cine MRI data in each subject; (Kudelka et al. 1997): (i) **early diastolic filling rate** (defined as the maximum value of the first derivative between end-systole and the diastolic midpoint), (ii) **late diastolic filling rate** (the maximum value of the first derivative between the midpoint and end-diastole), (iii) **E/A ratio** (the peak early rate divided by the peak late rate), and (iv) **early filling percentage** (the volume increase from end-systole to the midpoint divided by the stroke volume x 100).

Longitudinal shortening was determined from cine MRI in the 4-chamber view by determining the perpendicular distance from the plane of the mitral valve to the apex in systole and diastole. The eccentricity ratio was calculated as the left ventricular mass divided by the end-diastolic volume, as a measure of concentric remodelling (Cheng et al. 2009).

3.4.2 Cardiac tagging

Cardiac tagging works by applying radiofrequency pulses to cancel magnetic resonance signal from the myocardium, which appear as tags. The deformation of these tags can be tracked throughout the cardiac cycle. A turbo field echo sequence with acceleration factor 9 was used to collect short axis slices (TR/TE/FA/NEX = 4.9/3.1/10⁰/1, SENSE factor 2, FOV 350x350mm, voxel size 1.37mm, tag spacing 7mm). Short-axis slices of 10mm thickness were prescribed on the 2 and 4 chamber views as per (Lumens et al. 2006) (Figure 32) avoiding the base and apex due to through-plane motion during the cardiac cycle.

The Cardiac Image Modelling package (University of Auckland) was used to analyse the tagging data by aligning a mesh on the tags between the endo- and epicardial contours (Figure 33). Circumferential strain was calculated throughout the cardiac cycle and is quoted for both the whole myocardial wall and the endocardial third of the wall thickness at mid-ventricle. Cardiac torsion between the two planes (taken as the circumferential-longitudinal

shear angle defined on the epicardial surface), was calculated. Details of how this is calculated is described in section 1.5.2.

Figure 32 Tagging two parallel short axis sections 10mm apart (either side of the mid-ventricle (dotted line))

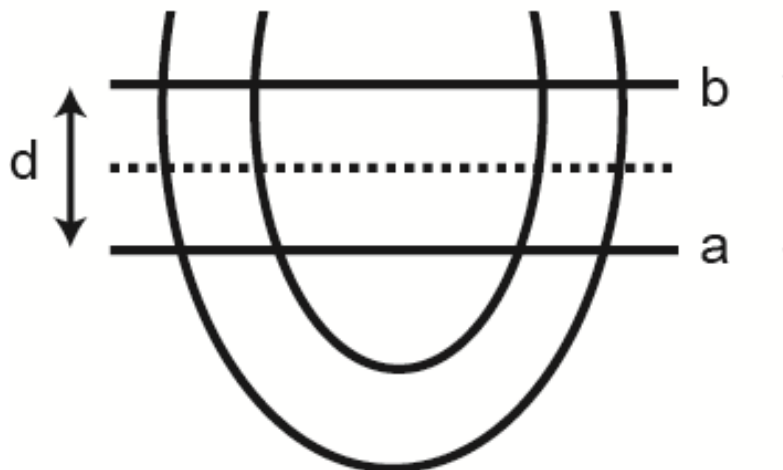
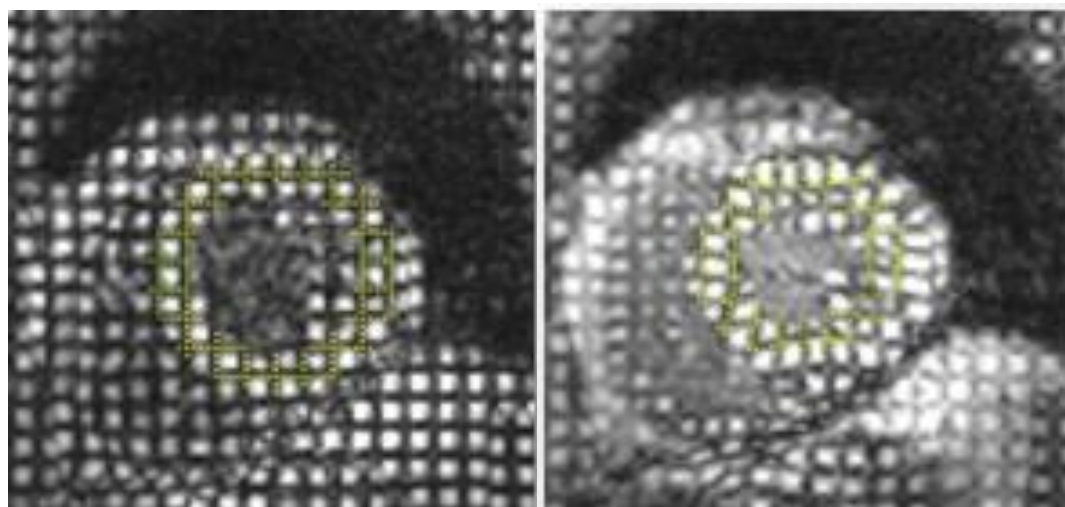


Figure 33 Mesh placed on the magnetic tags at (left) end-diastole and (right) end-systole



Torsion is a marker of dominance of the epicardial fibres over endocardial fibres. TSR quantifies this, and is a ratio of the shear angle between two planes on the epicardial surface (Lumens et al. 2006) and the peak circumferential strain in the endocardial third of the myocardium (Lumens et al. 2006; Van Der Toorn et al. 2002). The recoil of torsion in diastole occurs rapidly in early diastole and has been shown to correlate closely with the time constant of isovolumic relaxation derived from the left ventricular pressure

waveform (Dong et al. 2001). This was expressed as the torsion recoil rate (which is normalised for peak torsion, %/ms).

3.4.3 Cardiac spectroscopy

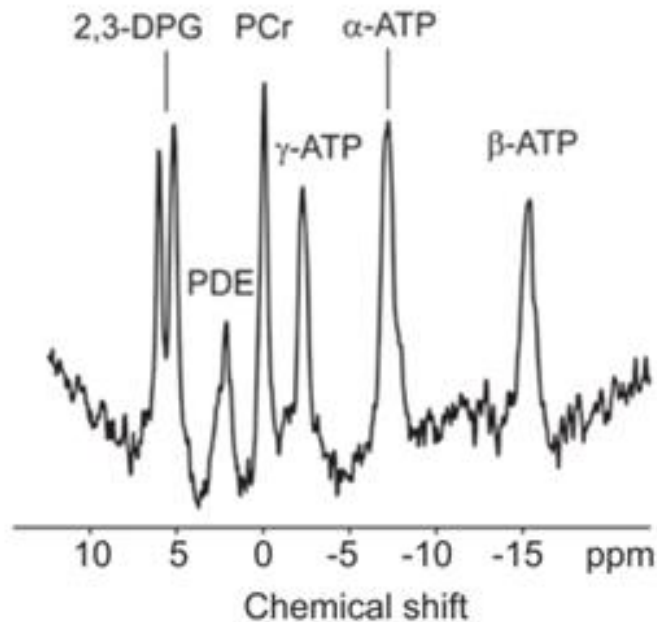
Cardiac high-energy phosphate metabolism was assessed using ^{31}P -MRS. With participants lying in a prone position with their heart at magnet isocentre, data was collected using a 10cm diameter ^{31}P surface coil (Pulseteq, UK) for transmission/reception of signal. A slice selective, cardiac-gated cardiac gated 1-dimensional chemical shift imaging (1D-CSI) sequence was used to eliminate contamination from the liver, with spatial pre-saturation of lateral skeletal muscle to avoid spectral contamination. Sixteen coronal phase-encoding steps were used, yielding spectra from 10-mm slices (TR = heart rate, 192 average, approx. 20-min acquisition time). Spectral locations were overlaid onto an anatomical image and the first spectrum arising entirely beyond the chest wall was selected. Details of our processing and correction of the spectra for blood contamination, saturation, excitation flip angle and analysis are below.

Negligible liver contamination was assured by 1-D foot-head oriented CSI experiments in phantoms, which showed that using the same coil and power settings, less than 1% of the total phosphorus signal originated from outside the prescribed area. The resonance and rf pulse frequencies were centred half-way between the two principal peaks of interest, PCr and γ -ATP. The excitation flip angle was set such that the excitation achieved at 65mm from the coil would be approximately 50 degrees, since the Ernst angle for maximum SNR for TR=1s (heart rate of 60bpm) would be 40° for PCr and 49° for γ -ATP based on their reported T_1 relaxation times at 3.0T (Tyler et al. 2008). The actual flip angle obtained at the depth of the region of interest was determined using a gadolinium-doped 20mM phenyl phosphonic acid phantom at the centre of the coil. Acquisition of five phantom spectra at 5°-45° nominal flip angle (TR=4s, NSA=4) allow the flip angle at the centre of the coil to be calculated and a B1 model based on (Haase et al. 1984), previously validated phantom experiments, is used to work out the flip angle

at the depth of interest. This can then be used to provide correction for T1 saturation effects.

Spatial pre-saturation of lateral skeletal muscle using a 25mm thick slab was used. Sixteen coronal phase-encoding steps yielded spectra from 10mm slices (TR=heart rate, 192 averages, acquisition time approximately 20 minutes), using a trigger delay of 400ms. A cosine apodization filter was applied. The first spectrum containing signal beyond the chest wall with signal solely from cardiac tissue was selected. The spectrum was analysed using an AMARES time domain fit in jMRUI (Vanhamme et al. 1999) to quantify PCr, the γ resonance of ATP and 2,3-diphosphoglycerate (DPG). ATP peak area was corrected for blood contamination by $1/6^{\text{th}}$ combined 2,3-DPG peak (Figure 34) (Conway et al. 1998; Bottomley 2007). It is important to note that cardiac energetics were taken from the anterior cardiac wall whereas structural and functional cardiac measures (described above) took into account the entire left ventricle.

Figure 34 Phosphorus spectroscopy from a control subject (PCr/ATP=1.95). Spectrum presented before correction for saturation due to blood content, flip angle at the cardiac tissue and heart rate



3.4.4 Cardiac MRI reliability

For some of the key cardiac MRI measures, intra-observer and inter-observer reliability were calculated. Intra-observer limits of agreement were 0.08 ± 0.16 for E/A ratio, $0.68 \pm 2.84\%$ for early filling percentage and $0.06 \pm 0.51^\circ$ for torsion. Inter-observer reliability limits of agreement were 0.06 ± 0.11 for E/A ratio, $0.4 \pm 1.58\%$ for early filling percentage, $14.84 \pm 10.30\text{ml}$ for end-diastolic volume and $5.44 \pm 6.08\%$ for ejection fraction.

These reliability measures are within respectable ranges, however the larger limits of agreement for inter-observer systolic and structural parameter reliability, means that where possible one observer will carry out all cardiac analysis.

3.4.5 Liver spectroscopy

Intrahepatic lipid was measured by localized T2-corrected ^1H -MRS at multiple echo times using the point-resolved spectroscopy (PRESS sequence: TR/TEs = 3000ms/36,50,75,100,125,150ms, voxel size 3x3x3cm)

placed in the posterior right lobe to avoid major vessels. One “large” voxel (compared to biopsy) was used which is better than using 2 or 3 smaller ones, as it improves the signal to noise ratio and minimizes patient time in the magnet (Szczepaniak et al. 2005). The water and fat resonances were analysed using jMRUI version 3.0. (Naressi et al. 2001). Following manual phase correction, spectra were analysed using a non-linear least squares algorithm (AMARES) (Vanhamme et al. 1999). Liver fat was corrected for proton density of water and lipid (Longo et al. 1995) using the following equation:

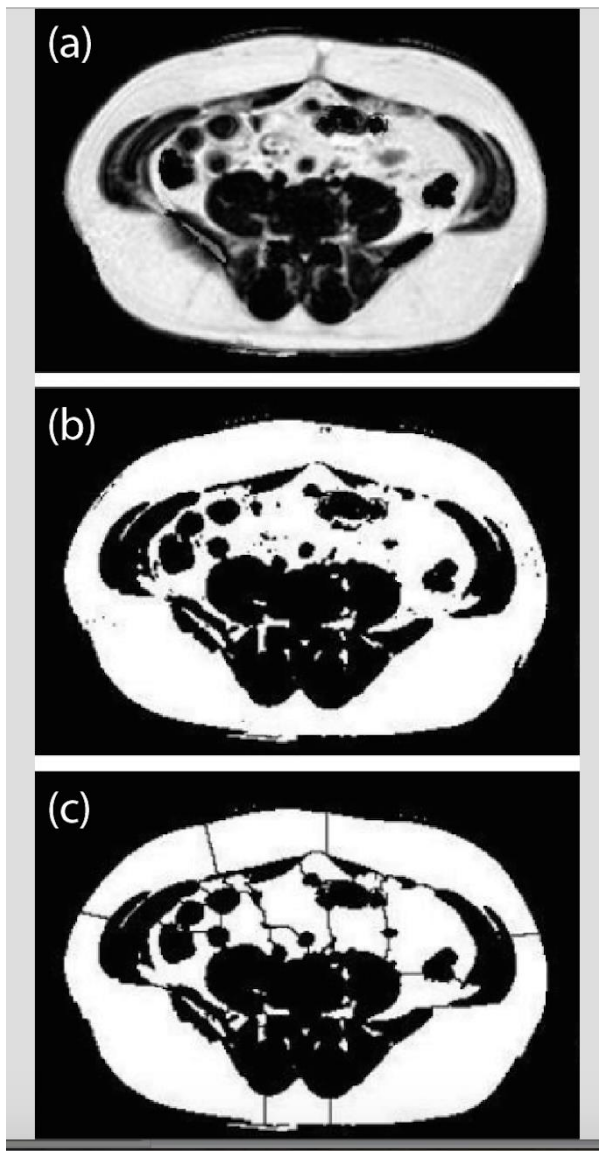
$$V_f = V_w (D_w/D_f) [FTSA / (N_v - FTSA)]$$

where V_f and V_w are the volumes of the fat and water phases, respectively, and D_f and D_w are the proton-density values of the fat and water phases, respectively. A D_w value of 111mol/L and D_f value of 110mol/L was used. FTSA is the ratio of the detectable fat signal peak area to the total signal peak area and N_v is the ratio between the signal integrals in the two spectral regions containing lipids (Longo et al. 1995).

3.4.6 Visceral and Abdominal fat

Subcutaneous and visceral fat content was acquired using images at the L4/L5 junction with a three-point Dixon sequence (Figure 35a) (TR/TE/number of averages/flip angle 50 ms/ 3.45, 4.60, 5.75 ms/1/308, matrix 1603109, median field of view (FOV) 440 mm, range 400e480mm to suit subject size with 70% phase FOV). Slices were 10mm thick and acquired during a breath-hold (Donnelly et al. 2003). Image J was used for analysis (Abramoff et al. 2004). Fat and water were separated, and binary gating applied, to produce a map of structures containing more than 50% fat (subcutaneous and visceral fat) from those with less. The binary image was divided into distinct areas using a watershed algorithm (Figure 35b). This allowed easy separation of the subcutaneous and visceral fat around the boundary of the chest wall. Selection of subcutaneous fat and any external signals allows measurement of this area, and subtraction from the total to yield visceral fat area (Figure 35c).

Figure 35 Quantifying visceral fat (a) L4/L5 image, (b) binary thresholding, (c) separation of visceral from subcutaneous fat



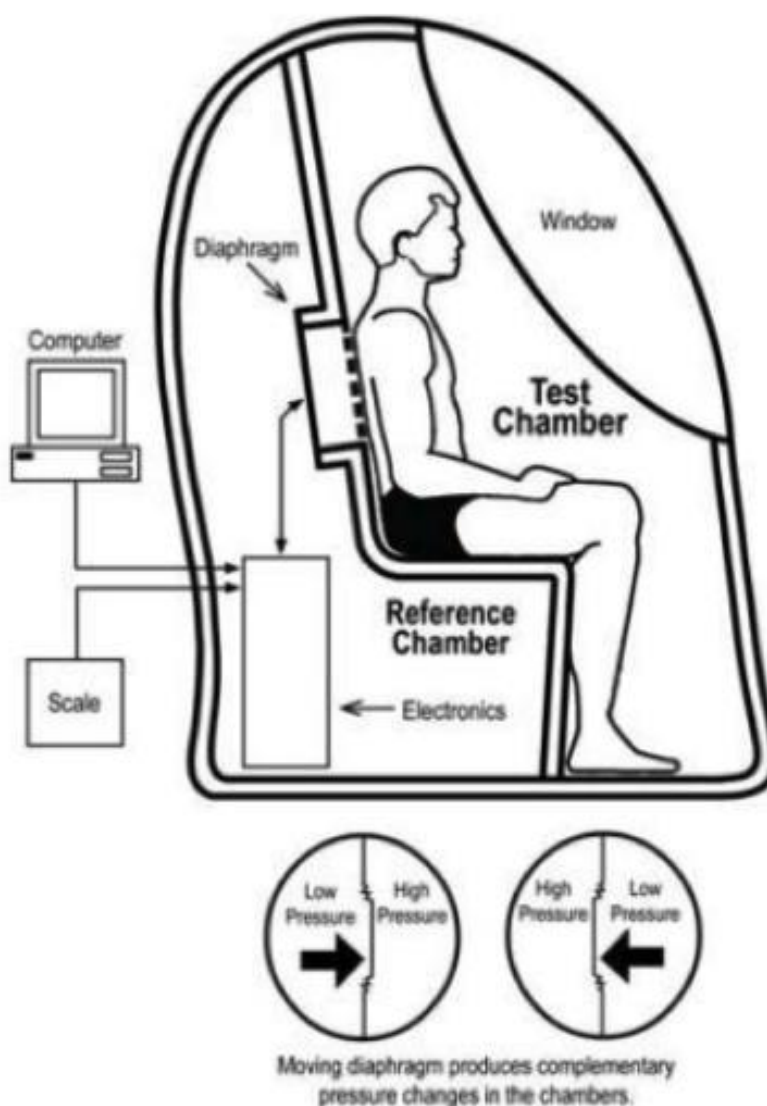
3.5 Body composition

Body composition was measured using air displacement plethysmography (Figure 36) (BodPod, Life Measurement Inc., CA, USA). Patients were asked to refrain from exercising, eating and drinking 2 hours prior to assessment and asked to wear tight fitting underwear or lycra along with a swimming cap to minimise air trapping. Patients were required to sit still and breathe normally for two 30 second bouts.

Body volume was measured indirectly by determining the change in pressure caused by the volume of air displaced by the subject sitting inside the chamber. The BODPOD is divided into two chambers; a test chamber for the

subject and a reference chamber which have volumes of 450 and 300L respectively (Figure 36). A diaphragm oscillates between the 2 chambers, and when the volume is increased in one chamber, it decreases by the same amount in the other chamber. The resulting change in pressure is measured by transducers. Body volume is calculated because 'Boyles law' states that volume and pressure are inversely related. Body density is then calculated (Density=Mass/Volume), and subsequently, because the density of fat and fat free mass are known, the relative proportions of fat and lean mass can be calculated. This technique has been validated against the gold standard technique, hydrodensitometry (Fields et al. 2000) and in 980 healthy men and women, the BODPOD demonstrated good test-retest reliability (Noreen & Lemon 2006). Same day test-retest reliability (coefficients of variation; 1.7% ± 1.1%) was not different to hydrodensitometry test-retest reliability (2.3 ± 1.9%) (McCrary et al. 1995).

Figure 36 How the BodPod machine works



3.6 Glucose control

Patients underwent an OGTT after a minimum 8 hours overnight fast. Upon arrival, a 22 gauge cannula was inserted into the cubital fossa and a fasting blood sample collected. A timer was initiated upon ingestion of 75g of glucose (equivalent to 394ml of Lucozade original 73kcal bottle) and 10ml blood samples were collected every 15 mins thereafter for 90 mins, after which a final blood sample was collected at 120mins. Samples were analysed immediately for whole blood glucose using the glucose oxidase method (YSI 2300 Stat Plus-D, Yellow Springs Instruments, Yellow Springs, OH). Glucose oxidase is an enzyme which reacts with glucose to produce

gluconic acid and hydrogen peroxide. Hydrogen peroxide passes through cellulose acetate to a platinum electrode where it is oxidized. The resultant current is proportional to the amount of glucose.

Blood samples for insulin were spun in a centrifuge (Harrier 18/80R; MSE Ltd, London, UK) at 3000rpm, 4°C for 10mins. Plasma was pipetted off each sample and stored at -40°C (Sanyo Biomedical freezer; Loughborough, UK). Insulin was batch-analysed (to increase intra-rater reliability and decrease inter-rater variability), using the Mercodia Iso-Insulin ELISA (cat no 10-1128-01, Mercodia, Sweden) in a clinical pathology accredited laboratory. Insulin sensitivity and β -cell function were estimated using the HOMA2 calculator (University of Oxford, 2013) (Levy et al. 1998). The HOMA2 equation uses measures of fasting glucose and insulin and simulates the physiological processes that influence these measures to predict β -cell function and insulin sensitivity. Glucose AUC was calculated using the trapezoidal rule (Floch et al. 1990).

3.7 Blood analysis

Fasting blood was collected in Gold and Lavender top BD vacutainer tubes for serum and whole blood haematology determinations, respectively, and sent down to a clinical pathology accredited laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry) for analysis. HbA_{1c} was analysed using HPLC on a TOSOH G8 (Minato, Tokyo, Japan) and all other biochemistry assays were analysed using a Roche Modular P800 (Basel, Switzerland); the methodologies are as follows-

ALP	Enzymatic Colourimetric
ALT	Enzymatic UV
Cholesterol	Enzymatic
GTT	Enzymatic
HDL	Enzymatic
Triglycerides	Enzymatic Colourimetric

**Chapter 4 Cardiac structure and function are altered in Type
2 diabetes and non-alcoholic fatty liver disease and
associate with glycaemic control**

4.1 Introduction

Type 2 diabetes affects ~5% of Western populations, with prevalence rates significantly higher in East and South Asian communities (IDF 2013). NAFLD is reported to affect between 20 and 30% of Western populations (Browning et al. 2004), can be as high as 60% in urban East and South Asian groups (Williams et al. 2011), and is closely related to the development of Type 2 diabetes (Sattar et al. 2007). Indeed, in excess of 90% of obese people with Type 2 diabetes have NAFLD (Tolman et al. 2007). The strong relationship between NAFLD and Type 2 diabetes lies in the central role of liver lipids in glucose homeostasis (Perry et al. 2014).

Heart disease is the leading cause of morbidity and mortality in *both* Type 2 diabetes and NAFLD (Garcia et al. 1974; Rafiq et al. 2009). Individuals with diabetes demonstrate a 74% greater risk of hospitalisation due to heart failure (Health and Social Care Information Centre 2013). NAFLD, characterized by elevated serum γ -glutamyltransferase (GGT), is independently associated with heart failure (Wang et al. 2013). The increased incidence of cardiovascular morbidity and mortality associated with Type 2 diabetes and NAFLD, has been linked to preclinical changes in cardiac structure, function and metabolism.

The most commonly reported change in asymptomatic individuals with Type 2 diabetes is diastolic dysfunction (Rijzewijk et al. 2009; Diamant et al. 2003; Venskutonyte et al. 2014; Graça et al. 2014), alongside decreased end-diastolic blood volume (Rijzewijk et al. 2009), and changes in cardiac strain patterns (Fonseca et al. 2004). These cardiac changes have been associated with myocardial steatosis (Korosoglou et al. 2012), mitochondrial dysfunction (Marciniak et al. 2014), changes in calcium regulation, and myocardial fibrosis (Lamberts et al. 2014). NAFLD is also characterized by a similar pattern of diastolic dysfunction (Goland et al. 2006; Pacifico et al. 2014), altered left ventricular geometry (Goland et al. 2006), reduced myocardial perfusion reserve (Nakamori et al. 2012), and changes in cardiac strain (Hallsworth et al. 2012). Although a growing body of epidemiological and clinical evidence links the disease processes of Type 2 diabetes and

NAFLD, little is known about how these conditions may differentially affect the heart.

4.1.1 Study aims

- 1) To compare the impact of Type 2 diabetes and NAFLD upon cardiac structure, function and metabolism.
- 2) To identify potential metabolic mediator of cardiac function.

4.2 Methods

4.2.1 Participants

In a case control study, 19 non-diabetic participants with NAFLD, 19 participants with Type 2 diabetes and 19 healthy controls, were compared for cardiac structure and function using MRI. This was an extension of a previous study (Hallsworth et al. 2012) in which NAFLD patients and healthy controls had already been recruited.

NAFLD patients were recruited into the study through Newcastle upon Tyne Hospitals NHS Foundation Trust. NAFLD was defined as >5% intrahepatic lipid on ¹H-MRS of the liver (section 3.4.5) with no evidence of advanced fibrosis (mean alanine aminotransferase / aspartate aminotransferase (ALT/AST) 0.82 ± 0.08). Patients with >5% intrahepatic lipid were excluded from the NAFLD group if they had a previous diagnosis of Type 2 diabetes, were on any glucose lowering medication, had an HbA_{1c} ≥ 48 mmol/mol or had any secondary causes of hepatic steatosis as listed in (Chalasani et al. 2012). 19 controls, matched for gender, were recruited from advertisements in local newspapers and were without hypertension, metabolic, liver or cardiac disease.

Nineteen Type 2 diabetes participants were recruited into the study (see section 3.1 for recruitment strategy and inclusion criteria). Written informed consent was obtained from each participant. The study protocol was approved by Newcastle and North Tyneside 1 Research Ethics Committee.

4.2.2 Screening visit

All participants underwent a medical history and full physical examination, and NAFLD and Type 2 diabetes patients underwent a cardiopulmonary exercise test to screen for any undiagnosed cardiac disease (see section 3.3 for details).

4.2.3 Fasting blood measures

Fasting blood samples were analyzed for whole blood glucose (YSI 2300 Stat Plus-D, Yellow Springs Instruments, Yellow Springs, OH). ALT, total cholesterol, triacylglycerols and HbA_{1c} were analysed in a clinical pathology

accredited laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry) (see sections 3.7 for blood analysis details).

4.2.4 Cardiac MRI

All participants underwent MRI measures in a 3.0 T Philips Intera Achieva scanner (Best, NL). Cardiac structure, function and energetics were measured by cine MRI, 2 dimensional tagging and ³¹P-MRS respectively (see section for 3.4 details).

Preload, afterload, contractility, and ventricular-arterial coupling were calculated from this data, in combination with blood pressure measurements. Preload was determined by the end-diastolic volume, afterload by arterial elastance [(E_a) = end-systolic pressure (systolic blood pressure x 0.9)/stroke volume (normalized to body surface area)], contractility by end-systolic elastance [(E_{es}) = end-systolic pressure/end-systolic volume (normalized to body surface area)], and ventricular-arterial coupling by the ratio of E_{es}/E_a.

4.2.5 Liver and visceral MRI

Liver fat was assessed by ¹H-MRS and visceral fat was estimated at the L4/L5 junction using a three point Dixon sequence (see section 3.4 for details). All cardiac, liver and visceral MRI analysis was performed by Sophie Cassidy who was blinded to group allocation.

4.2.6 Statistical analysis

Data are presented as means ± SD unless otherwise stated. All statistical tests were two-sided and performed using SPSS version 19 (IBM, NY, US). Continuous data were tested for normality using the Shapiro-Wilk test. Between group differences were evaluated using a one-way ANOVA with Bonferroni correction methods for multiple comparisons and a non-parametric alternative (Kruskal Wallis) for non-normally distributed data. Spearman's rank correlation was used to observe any relationship between metabolic parameters and cardiac parameters. Any significant relationships were then entered into a multiple linear regression model, adjusting for age, systolic blood pressure and anthropometry (BMI, body surface area, weight, systolic blood pressure). The goal of these analyses was to determine which

factors were responsible for the differences in cardiac structure and function between groups. P values <0.05 were considered statistically significant.

4.3 Results

Table 5 summarises the demographic data of the three groups. Body weight, BMI and systolic blood pressure were significantly higher in Type 2 diabetes and NAFLD compared with controls ($p < 0.05$). Both NAFLD and Type 2 diabetes demonstrated increased liver fat (9.4 ± 4.3 vs. 7.9 ± 6.7 vs. 2.5 ± 0.9 %; $p < 0.05$) while fasting glucose was higher in Type 2 diabetes only (7.2 ± 1.4 mmol/L; $p < 0.05$).

Table 5 Demographic and metabolic characteristics of study population by group

Variable	Controls (n=19)	NAFLD (n=19)	Type 2 diabetes (n=19)	P value
Age (yearr)	56 ± 14	54 ± 15	62 ± 8	0.127
Gender (men:women)	11:8	11:8	11:8	-
Height (cm)	169 ± 11	169 ± 9	168 ± 9	0.877
Weight (kg)	78 ± 11	83 ± 14	91 ± 14*	0.013
BMI (kg/m ²)	28 ± 4	29 ± 3*	33 ± 5*	0.012
Body surface area (m ²)	1.9 ± 0.2	1.9 ± 0.2	2.0 ± 0.2*	0.018
Visceral adipose tissue (cm ²)	-	154 ± 47	191 ± 75	0.120
Systolic blood pressure (mmHg)	131 ± 11	146 ± 16*	145 ± 17*	0.003
Diastolic blood pressure (mmHg)	82 ± 8	90 ± 12	89 ± 12	0.096
VO _{2peak} (ml min ⁻¹ kg ⁻¹)	-	24 ± 6	19 ± 5†	0.007
Fasting Glucose (mmol/L)	5.2 ± 0.5	5.0 ± 0.6	7.2 ± 1.4*†	0.000
HbA _{1c} (mmol/mol) (%)	-	38 ± 5 (5.6 ± 0.4)	58 ± 10† (7.4 ± 0.9)	0.000
Intrahepatic lipid (%)	2.5 ± 0.9	9.4 ± 4.3*	7.9 ± 6.7*	0.000
ALT (U.L)	23 ± 12	51 ± 39*	30 ± 11	0.013
Total Cholesterol (mmol/L)	5.3 ± 0.7	5.1 ± 1.2	4.7 ± 1.4	0.342
Triglycerides (mmol/L)	1.7 ± 0.9	1.5 ± 0.8	1.3 ± 1.1	0.328
Medications				-

Statins	0	4	10	
Blood pressure	0	1	8	
Metformin	0	0	12	

Data are means \pm SD.

*Significant difference disease vs. control ($p < 0.05$).

† Significant difference Type 2 diabetes vs. NAFLD ($p < 0.05$).

VO_{2peak} was significantly lower in the Type 2 diabetes group compared to NAFLD ($p < 0.01$). There was no significant difference in visceral adipose tissue ($p = 0.120$), blood cholesterol ($p = 0.342$) or triglycerides between patients and controls ($p = 0.328$, Table 5), however it should be noted that both Type 2 diabetes and NAFLD patients were taking lipid-lowering medication.

4.3.1 Cardiac structure and systolic function

Left ventricular mass was similar in all groups ($p = 0.581$). The NAFLD group demonstrated thicker walls at end-systole and end-diastole ($p < 0.05$). Cardiac structural concentric remodelling was observed in both NAFLD and Type 2 diabetes, as shown with an increased eccentricity ratio (1.12 ± 0.2 vs. 1.05 ± 0.3 vs. 0.89 ± 0.2 g/ml; $p < 0.05$) and reduced end-diastolic volume indexed when compared with healthy controls ($p < 0.05$) (Table 6, Figure 37a). An increased eccentricity ratio was associated with diastolic dysfunction in NAFLD (E/A: $r = -0.4$, $p = 0.05$) and Type 2 diabetes (E/A: $r = -0.56$, $p = 0.012$; Early filling rate: $r = -0.59$, $p = 0.009$; Early filling %; $r = -0.64$, $p = 0.01$) but not in the control group.

Systolic function was impaired in the Type 2 diabetes group, evidenced by a lower stroke index (31 ± 7 vs. 38 ± 10 ml/m²; $p < 0.05$) and reduced longitudinal shortening (13.7 ± 4 vs. 16.6 ± 2.8 %; $p < 0.05$) when compared to controls (Figure 37b). There were no differences in heart rate, stroke volume, cardiac output and ejection fraction between groups and no significant correlations between measures of structural parameters and systolic function with fasting glucose or HbA_{1c}. Arterial elastance (afterload) and ventricular elastance (ventricular stiffness) were both increased in NAFLD and Type 2 diabetes compared to controls ($p < 0.05$) but the ratio between the two (ventricular-arterial coupling) was not different between groups (Table 6)

Table 6 Magnetic resonance imaging measurements of cardiac structure, function and metabolism

	Controls (n=19)	NAFLD (n=19)	Type 2 diabetes (n=19)	P value
Cardiac structure				
Left ventricular mass (g)	102 ± 26	114 ± 31	108 ± 28	0.581
Left ventricular mass indexed (g/m ²)	55 ± 12	59 ± 11	53 ± 12	0.273
Wall thickness diastole (mm)	7 ± 1	8 ± 1*	6 ± 2*†	0.000
Wall thickness systole (mm)	12 ± 2	14 ± 3*	12 ± 3	0.016
Eccentricity ratio (g/ml)	0.89 ± 0.2	1.12 ± 0.2*	1.05 ± 0.3*	0.004
End-diastolic volume indexed (ml/m ²)	64 ± 18	54 ± 14	52 ± 14	0.039
End-systolic volume indexed (ml/m ²)	27 ± 9	21 ± 9	21 ± 10	0.63
Systolic function				
Heart rate (bpm)	59 ± 9	61 ± 9	65 ± 9	0.178
Stroke volume (ml)	70 ± 19	64 ± 12	64 ± 17	0.437
Stroke index (ml/m ²)	38 ± 10	33 ± 5	31 ± 7*	0.034
Cardiac output (L/min)	4.0 ± 0.8	3.8 ± 0.6	4.0 ± 0.9	0.754
Ejection fraction (%)	59 ± 5	63 ± 8	61 ± 10	0.332
Longitudinal shortening (%)	16.6 ± 2.8	14.2 ± 2.7	13.7 ± 4*	0.017
Arterial elastance	3.32 ± 0.85	4.07 ± 0.78*	4.38 ± 1.05*	0.004
Ventricular elastance	5.04 ± 2.05	7.62 ± 3.22*	7.72 ± 4.08*	0.011
Ventricular-arterial coupling	1.50 ± 0.35	1.82 ± 0.60	1.75 ± 0.72	0.263
Diastolic function				
Early filling percentage (%)	69 ± 11	65 ± 11	57 ± 9*	0.003
E/A	1.9 ± 1.4	1.6 ± 1.3	0.9 ± 0.4*	0.015
Early diastolic filling rate (ml/s)	312 ± 121	265 ± 95	244 ± 76	0.105
Late diastolic filling rate (ml/s)	203 ± 73	212 ± 70*	288 ± 99*	0.009
Strain and torsion				
Peak endocardial circumferential strain (%)	22 ± 5	28 ± 4*	24 ± 5†	0.001

Peak whole wall circumferential strain (%)	17 ± 3	19 ± 2	16 ± 4†	0.012
Peak torsion (°)	6.6 ± 1.8	6.9 ± 2.2	8.0 ± 2.5	0.127
Torsion recoil rate (%/ms)	0.25 ± 0.12	0.17 ± 0.12	0.27 ± 0.1†	0.017
Torsion to shortening ratio	0.51 ± 0.15	0.44 ± 0.13	0.58 ± 0.16†	0.019
Metabolism				
PCr/ATP ratio	1.9 ± 0.3	1.8 ± 0.3	1.8 ± 0.3	0.543

Data are means ± SD.

*Significant difference disease vs. control (p<0.05).

† Significant difference Type 2 diabetes vs. NAFLD (p<0.05).

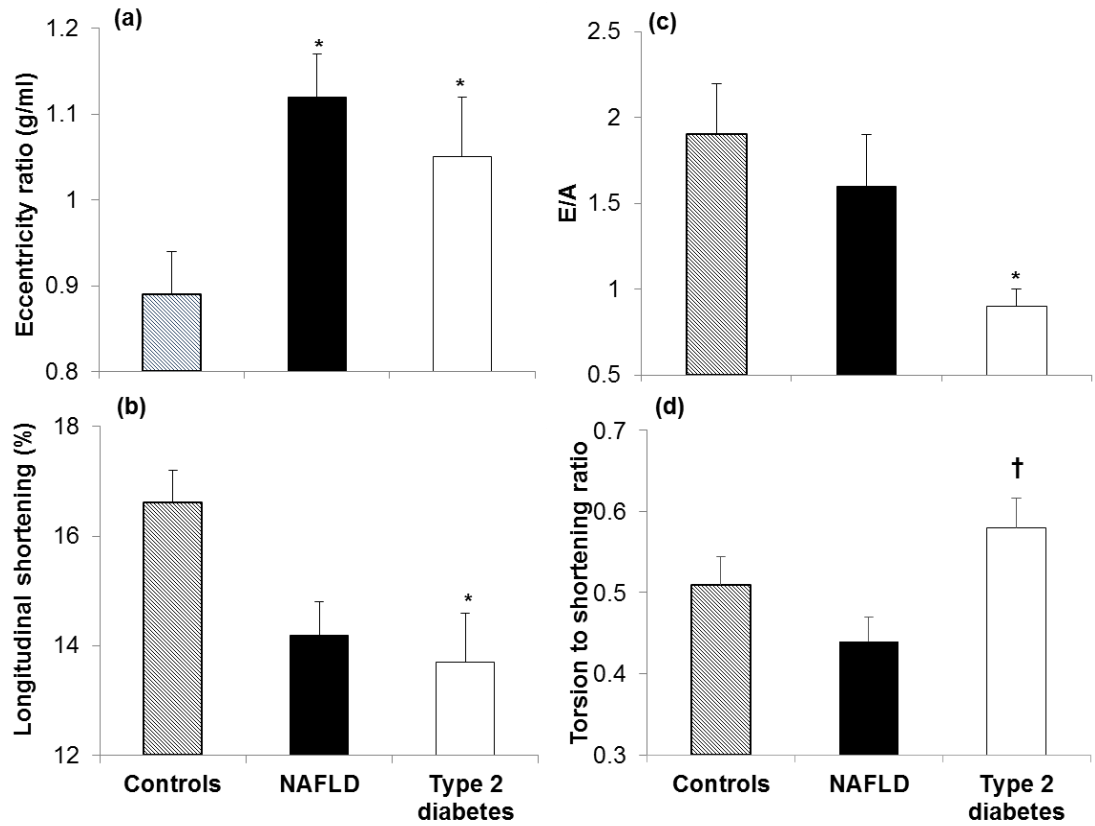
4.3.2 Diastolic function

E/A was significantly lower in Type 2 diabetes compared to controls (0.9 ± 0.4 vs 1.9 ± 1.4 ; $p < 0.05$) (Figure 37c) along with a decrease in early filling percentage ($p < 0.05$). The NAFLD group showed no significant change in these parameters compared to controls. Both the NAFLD and Type 2 diabetes groups displayed significant increases in late diastolic filling rate compared to controls (212 ± 70 vs. 288 ± 99 vs. 203 ± 73 ml/s; $p < 0.05$). Across the three groups, there was a moderate negative correlation between fasting glucose and early filling percentage ($r = -0.32$, $p = 0.021$) (Figure 38a). In addition, increased HbA_{1c} was associated with impaired E/A ($r = -0.52$, $p = 0.003$) and reduced early filling rate ($r = -.48$, $p = 0.006$) (Figure 38c+d). When controlling for the baseline differences in age, systolic blood pressure and anthropometry across groups; glucose ($\beta = -0.26$, $p < 0.05$) remained a significant predictor of early filling percentage. Age was a significant predictor of early filling percentage ($\beta = -0.5$, $p < 0.01$), E/A ($\beta = -0.66$, $p < 0.01$) and early filling rate ($\beta = -0.48$, $p < 0.01$).

4.3.3 Cardiac torsion and strain

Cardiac torsion and strain differed across groups, with higher endocardial circumferential strain in the NAFLD group and elevated torsion in the Type 2 diabetes group, as shown by the significantly higher TSR in Type 2 diabetes compared to NAFLD (0.58 ± 0.16 vs. 0.44 ± 0.13 , $p < 0.05$, Figure 37d). Peak torsion was 24% greater in the Type 2 diabetes group compared to controls. Fasting glucose was moderately correlated with TSR ($r = .42$, $p = 0.003$) (Figure 38b). When controlling for baseline differences in age, systolic blood pressure and anthropometry; the association between fasting glucose and TSR was approaching significance ($\beta = 0.25$, $p = 0.072$). Similarly, age was approaching significance in predicting the torsion to shortening ratio ($\beta = 0.29$, $p = 0.057$).

Figure 37 Measures of cardiac structure and function, namely, (a) eccentricity ratio, (b) longitudinal shortening (c) E/A and (d) torsion to shortening ratio, in control, NAFLD and Type 2 diabetes adults.



Data are means \pm SE.

*Significant difference disease vs. control ($p < 0.05$).

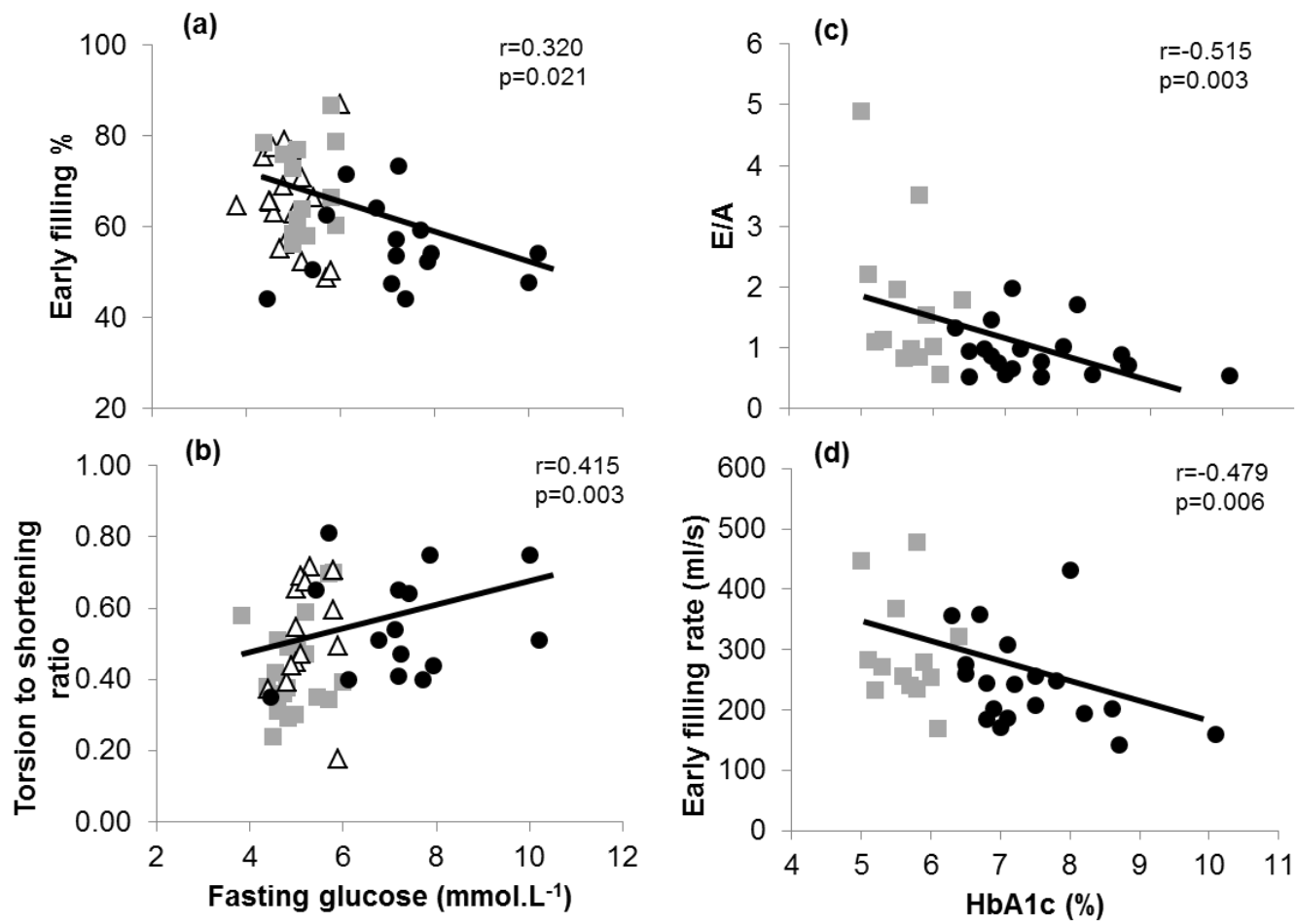
† Significant difference Type 2 diabetes vs. NAFLD ($p < 0.05$)

4.3.4 Cardiac metabolism

There was no difference in PCr/ATP amongst NAFLD and Type 2 diabetes adults compared to controls (1.8 ± 0.3 vs. 1.8 ± 0.3 vs. 1.9 ± 0.3 ; $p = 0.543$) and PCr/ATP correlated with no measures of cardiac structure or function.

Figure 38

Associations between glycemic control and measures of cardiac function. *Triangle= Control, Square= NAFLD, Circle=Type 2 diabetes.* Relationships between (a) fasting glucose and early filling percentage, (b) fasting glucose and torsion to shortening ratio, (c) HbA_{1c} and E/A and (d) HbA_{1c} and early filling rate, are presented in the figure



4.4 Discussion

This is the first study to compare the effect of Type 2 diabetes and NAFLD on cardiac structure, function and metabolism using the most sensitive cardiac MRI techniques. The major findings suggest that despite similar levels of concentric remodelling, individuals with Type 2 diabetes demonstrate significantly greater diastolic and subendocardial dysfunction in comparison with NAFLD and healthy adults. There were significant relationships between glycaemic control and measures of cardiac function, suggesting that hyperglycaemia itself is an important factor contributing to these sub-clinical cardiac changes.

The results indicate concentric remodelling in both NAFLD and Type 2 diabetes independent of changes in left ventricular wall mass. Using similar magnetic resonance techniques, Diamant et al. (Diamant et al. 2003) also failed to demonstrate an increase in left ventricular wall mass in adults with Type 2 diabetes which is in contrast to studies using echocardiography (Vanninen et al. 1992). Echocardiography has however been found to overestimate cardiac size (Missouris et al. 1996). In both NAFLD and Type 2 diabetes, a raised eccentricity ratio was associated with diastolic dysfunction. We speculate that the reduced left ventricular cavity may impair diastolic filling. The NAFLD group display more prominent structural changes than the Type 2 diabetes group, with thicker walls at diastole and systole. This enables the left ventricle to generate increased force and pressure thereby preserving systolic function (Adeghate & Singh 2014).

The data show left ventricular diastolic and systolic dysfunction in Type 2 diabetes but not NAFLD, despite equivalent degrees of blood pressure and concentric remodelling. Early diastolic filling due to ventricular relaxation accounts for roughly 80% of ventricular end-diastolic volume in a young healthy heart and declines with age (Hollingsworth et al. 2012). Impaired early filling observed in the present study indicates greater myocardial stiffness. Diastolic dysfunction is an independent predictor of mortality (Halley et al. 2011) which warrants the need for therapies to target these

preclinical cardiac changes. A recent longitudinal study demonstrated no decline in diastolic dysfunction after 6 years of follow up in adults with Type 2 diabetes, when cardiovascular risk factors (such as blood pressure, BMI and blood glucose) were managed (Venskutonyte et al. 2014). Interventions targeting these risk factors therefore need to be a priority in the treatment of diastolic dysfunction. The present study uses MRI in contrast to the aforementioned study and many other reports in the literature. MRI provides a robust non-invasive assessment of cardiac function and is considered the gold standard for measures of cardiac structure, enabling the measurement of cardiac changes in a unique non-invasive way.

Analysis of cardiac deformation by 3D magnetic resonance tagging demonstrated a raised TSR in Type 2 diabetes with peak torsion 24% greater in this group. A decrease in circumferential and longitudinal strain accompanied with a rise in torsion has been previously reported in Type 2 diabetes (Fonseca et al. 2004). TSR is a marker of the dominance of subepicardial fibres exerting their effects over the subendocardium (Lumens et al. 2006). Torsion is a normal feature of cardiac contraction and results in a counter clockwise twisting motion when viewed from the apex to base. Subepicardial fibres act over a larger radius, therefore, during contraction they are dominant over subendocardial fibres which partially counteract this twisting motion (Lumens et al. 2006). The relative dysfunction of the subendocardium in Type 2 diabetes, manifested as an increase in torsion, could be attributed to subendocardial fibrosis (Lumens et al. 2006). Subendocardial dysfunction reduces longitudinal shortening as demonstrated in this study. In contrast to Type 2 diabetes, NAFLD participants demonstrate increased strain and maintained torsion when compared to controls. The large difference in radii between epicardial and endocardial fibres (due to both increased wall thickness and reduced end-diastolic blood volume indexed) in NAFLD, means endocardial strain has to increase for torsion to be maintained.

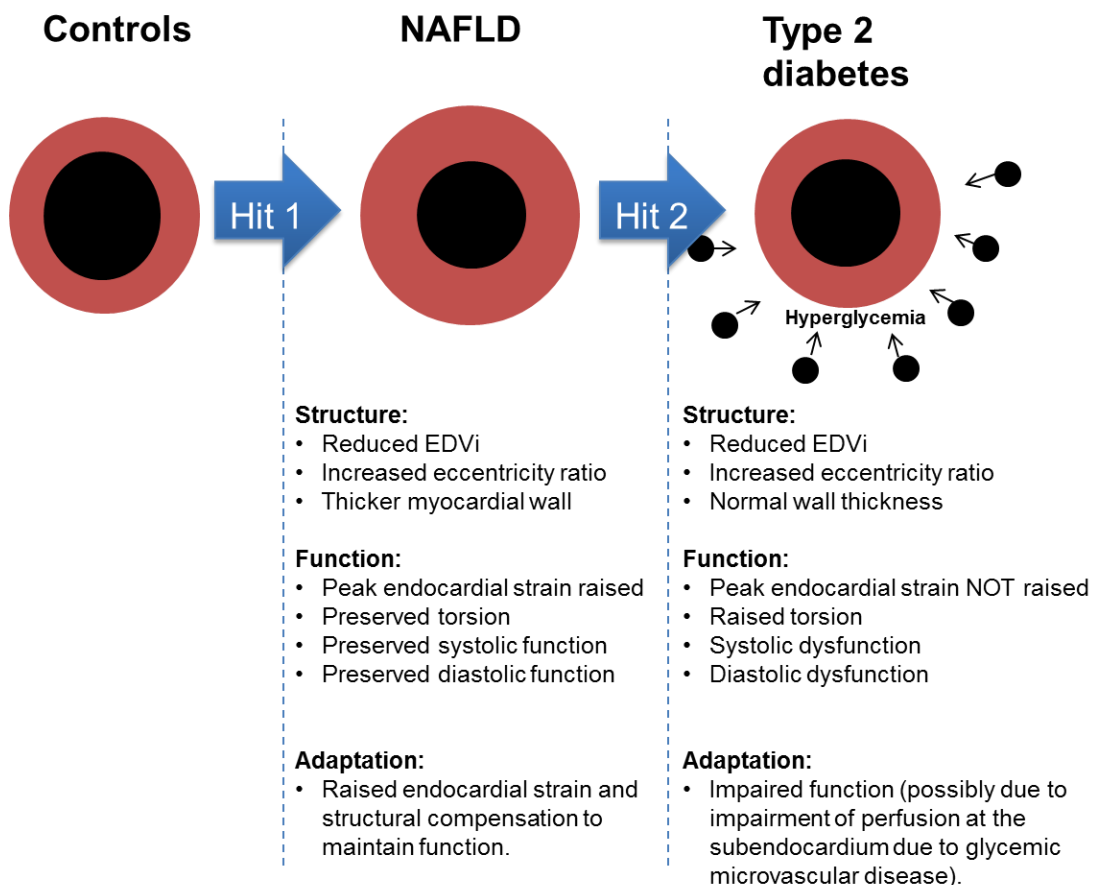
Across the three groups, age and fasting blood glucose were predictors of the changes in cardiac function. It has been previously demonstrated that age is associated with diastolic dysfunction and an increase in TSR (Lumens

et al. 2006; Hollingsworth et al. 2012; Ichikawa et al. 2013), and our results confirm these findings. Despite this, fasting blood glucose independent of age, influenced cardiac function, suggesting that metabolic disease exaggerates the ageing phenotype. The relative contribution of liver fat and other metabolic parameters (BMI, systolic blood pressure, total cholesterol, triacylglyceride) with cardiac complications is an important question. In the linear regression model, there were no consistent predictors of cardiac dysfunction other than blood glucose. However, to fully explore this question larger studies are required. The relationship with blood glucose is of interest. We speculate that NAFLD participants who have high liver fat with stable blood glucose demonstrate higher endocardial strain and structural compensation to maintain cardiac function. The progression to Type 2 diabetes, which is characterised by high blood glucose, may lead to endocardial damage, resulting in impaired function. This is reflected in the significant relationship between blood glucose and measures of cardiac function across the three groups. Preventing a rise in blood glucose should therefore be a priority in the clinical management of NAFLD. The postulated interactions between cardiac parameters in NAFLD and Type 2 diabetes are summarised in Figure 39.

It has been previously demonstrated that poor glycaemic control is associated with an increased risk of heart failure in adults with Type 2 diabetes (Iribarren et al. 2001). The present study builds on this by demonstrating an association between glycaemic control and changes in myocardial function. The relationship between cardiac torsion and glycaemic control has not been previously shown and only a few studies have demonstrated a relationship between glucose control and diastolic function (Korosoglou et al. 2012; Rijzewijk et al. 2009). Direct primary effects on the myocardium or secondary effects on peripheral resistance (afterload) are two pathways in which blood glucose could interfere with diastolic function. This is because diastolic distensibility (raised diastolic pressure at any level of diastolic volume) can arise from altered myocardial elastic properties (fibrosis) or prolongation of ventricular relaxation (Bonow & Udelson 1992). Raised afterload slows ventricular relaxation which can alter the pressure gradient

required during early diastolic filling and elevations in afterload have been shown to induce left ventricular diastolic dysfunction (Leite-Moreira et al. 1999). In this study, arterial elastance (afterload) was increased in Type 2 diabetes and NAFLD, but was not associated with glucose control. This is suggestive of a direct impact of glucose on the myocardium rather than peripheral resistance. It has been recently shown that subclinical myocardial damage occurs in those with pre-diabetes too, those with myocardial damage were at highest risk of mortality and cardiovascular events, particularly heart failure (Selvin et al. 2014). Despite clear associations, a causal relationship between blood glucose and cardiac complications cannot be inferred from this study, and more work is needed to identify the direct impact of hyperglycaemia on the heart.

Figure 39 Postulated interactions between cardiac parameters across controls, NAFLD and Type 2 diabetes (EDVi, end-diastolic volume indexed)



Despite changes in structure and function, cardiac high energy phosphate metabolism was similar between the three groups. A PCr/ATP ratio reduction

of 35% has previously been demonstrated in participants with Type 2 diabetes (Scheuermann-Freestone et al. 2003) and a 13% reduction in NAFLD, compared to healthy controls (Perseghin et al. 2008). The reduction in PCr/ATP ratio has also been correlated with diastolic dysfunction in people with Type 2 diabetes leading the authors to postulate increased concentration of NEFA in metabolic disease causes a switch from glucose to lipid cardiac metabolism, reducing efficiency of ATP production and causing cardiac functional changes (Diamant et al. 2003). However, the characteristics of the Type 2 diabetes participants in the aforementioned study were different from those in the present study. Specifically, they were not taking lipid-lowering medication and therefore had higher levels of triacylglyceride and total cholesterol values. Indeed, when participants were following current NICE guidelines (NICE 2014) and using lipid-lowering medications, the PCr/ATP ratio between Type 2 diabetes and controls were comparable (Rijzewijk et al. 2009). In the present study, triglyceride and cholesterol levels were similar in Type 2 diabetes, NAFLD and controls which could explain the lack of secondary difference in cardiac metabolism. These data also suggest that changes in high energy phosphate metabolism may reflect differences in substrate oxidation / supply rather than an underlying metabolic defect in the myocardium.

Limitations of the study should be considered. The cross sectional nature does not allow insight into causality of the abnormalities identified. Hypertension is a common comorbidity in metabolic disease which complicates the distinction of the separate impact of glucose control and high blood pressure on cardiac function. However, blood glucose had a significant relationship with measures of cardiac function independent of blood pressure. We did not measure perfusion or steatosis, two mediators of metabolism and function, due to the duration of MRI scans and tolerability by patients. Myocardial triacylglyceride accumulation is an early sign of heart disease in Type 2 diabetes and is associated with changes in cardiac function, in particular diastolic dysfunction (Rijzewijk et al. 2008; Ng et al. 2010; Korosoglou et al. 2012). The present data reinforces the need for further exploration of the interrelationship between, glycaemic control, cardiac

function, metabolism, perfusion, and steatosis. In addition, we were unable to assess stress MRI meaning cardiac abnormalities have only been identified at rest.

4.5 Conclusions

In summary, changes in cardiac structure are evident in adults with Type 2 diabetes and NAFLD without overt cardiac disease and without changes in cardiac energy metabolism. The growing prevalence of metabolic disorders puts large numbers at risk of these underlying cardiac changes. Only the Type 2 diabetes group display diastolic and subendocardial dysfunction and glycaemic control may be a key mediator of these cardiac changes.

Managing blood glucose should therefore be a priority for clinical care teams to prevent cardiac complications in adults with Type 2 diabetes and NAFLD.

The next section of the thesis will discuss the impact of a novel exercise intervention, designed to target these preclinical cardiac changes in adults with Type 2 diabetes.

Chapter 5 High intensity intermittent exercise improves cardiac structure and function and reduces liver fat in patients with Type 2 diabetes; a randomised controlled trial.

5.1 Introduction

Heart disease is the leading cause of morbidity and mortality in Type 2 diabetes (IDF 2013) and more than a quarter of all hospital admissions for heart failure in the West involve a patient with diabetes (Reis et al. 1997; Health and Social Care Information Centre 2013). Early changes in left ventricular structure and function have been identified in adults with Type 2 diabetes prior to any overt cardiac disease. These include; pathological hypertrophy (Dawson et al. 2005), reduced end-diastolic blood volume (Rijzewijk et al. 2009), diastolic and systolic dysfunction (Rijzewijk et al. 2009; Cassidy et al. 2015; Diamant et al. 2003) and alterations in strain patterns (Fonseca et al. 2004; Cassidy et al. 2015), identified using sensitive magnetic resonance techniques. A number of factors have been attributed to these changes including, protein glycation (Bodiga et al. 2013), myocardial steatosis (Korosoglou et al. 2012), myocardial fibrosis (van Heerebeek et al. 2008) and subendocardial perfusion deficits (Fischer et al. 1979; Lumens et al. 2006). MRI measurement has been shown to have greater reproducibility than 2 dimensional echocardiography in healthy and failing hearts, while avoiding the ionising radiation exposure of computerized tomography methods, which permits ethical longitudinal studies (Grothues et al. 2002). Despite clear cardiac dysfunction in Type 2 diabetes, therapies to target these preclinical cardiac changes are sparse.

Treatment algorithms for Type 2 diabetes support a physically active lifestyle at every stage of treatment (Inzucchi et al. 2012), indeed aerobic and resistance exercise have known benefits to cardiovascular function (Chudyk & Petrella 2011), yet little is known about the impacts upon cardiac structure and function. More recently, attention has been given to the intensity of exercise, with HIIT fast becoming a popular alternative to continuous moderate training (Gibala et al. 2012). HIIT refers to brief intervals of vigorous activity interspersed with periods of low activity or rest (Gibala et al. 2012) and not only reduces time commitment but is perceived to be more enjoyable than moderate continuous exercise (Bartlett et al. 2011). HIIT is known to elicit a strong cardiac response (Wisløff et al. 2007) but its potential

to improve cardiac structure and function in Type 2 diabetes is yet to be defined.

While the cardiac benefits of HIIT are still to be determined, the metabolic effects in Type 2 diabetes remain unclear also. Studies have demonstrated acute reductions in postprandial glycaemia after 1 (Gillen et al. 2012), or 6 sessions of HIIT (Little et al. 2011). Two 12 week HIIT studies have shown improvements in HbA_{1c}, one demonstrating a 0.4% reduction (Hollekim-Strand et al. 2014) and the other included southeast Asian patients (Mitranun et al. 2014), a group which have a genetic predisposition towards Type 2 diabetes (Hu 2011), and therefore cannot be generalised to Caucasian populations. Ectopic fat plays an important role in glucose homeostasis (Björntorp 1991; Taylor 2013), yet the impact of HIIT on regional fat deposition has not been investigated. Although HIIT is suggested to cause similar, if not superior physiological benefits compared to continuous exercise training (Hollekim-Strand et al. 2014), the cardiac and metabolic impact of HIIT are yet to be determined before it can be recommended for use by people with Type 2 diabetes.

5.2 Study aims

- 1) The primary aim of this study was to investigate HIIT as a potential therapy to improve cardiac structure and function in Type 2 diabetes.
- 2) The secondary aims of the study were to explore the impact of HIIT on glycaemic control and regional fat deposition.

We hypothesised that HIIT would improve cardiac structure and function, alongside improving glycaemic control and reducing ectopic fat in adults with Type 2 diabetes.

5.3 Methods

5.3.1 Participants

Twenty-eight adults with Type 2 diabetes were recruited into the study (see section 3.1 for recruitment strategy and inclusion criteria). Written informed consent was obtained from each participant. The study protocol was approved by Newcastle and North Tyneside 1 Research Ethics Committee.

5.3.2 Experimental protocol + randomisation

Following an initial screening visit; cardiac structure and function, liver and visceral fat, body composition, glycaemic control, resting and exercise metabolism, cardiac function during exercise and blood parameters were measured at baseline and after 12 weeks of HIIT or continued standard care. Patients were randomised into groups using a simple random allocation sequence (www.randomization.com). Concealed envelopes with subsequent numbers were locked in a drawer and withdrawn consecutively by Sophie Cassidy. All study procedures are described below, followed by a description of the intervention.

5.3.3 Screening visit

This visit only took place at baseline (not after the intervention). A medical history, physical examination and cardiopulmonary exercise test were used to screen for any underlying cardiac disease or contraindications to taking part in the intervention (see section 3.3 for details).

5.3.4 Magnetic resonance imaging

A 3.0 Tesla Philips Intera Achieva scanner (Best, NL) was used for all MRI examinations. Cardiac structure, function and energetics were measured by cine magnetic resonance imaging, cardiac tagging and ³¹P-Magnetic resonance spectroscopy respectively (see section 3.4 for details).

Liver fat was assessed by ¹H-MRS and Visceral fat was estimated at the L4/L5 junction using a three point Dixon sequence (see section 3.4.6 for details). All MRI analysis was performed by Sophie Cassidy who was blinded to group allocation.

5.3.5 Body composition

Body composition was measured using air displacement plethysmography to see the change in body fat after the intervention (see section 3.5 for details).

5.3.6 Glycemic control and blood analysis

An OGTT was performed after an 8 hour minimum fast (see section 3.6 for details). From this test, fasting glucose, 2 hour glucose, glucose AUC were measured, alongside IR and β -cell function from HOMA 2. The post intervention OGTT was performed within 48-72 hours of the final exercise session to control for the acute effect of exercise on glucose uptake.

Fasting bloods were also sent to a clinical pathology laboratory for measurement of ALT, AST, alkaline phosphatase (ALP), total cholesterol and triacylglycerol (see section 3.7 for details).

5.3.7 Resting blood pressure

During a 20 min resting period while patients lay supine in a quiet room without speaking or sleeping, beat-to-beat blood pressure was measured by the vascular unloading technique (Fortin et al. 1998) corrected automatically to the oscillometric blood pressure measured on the contralateral arm. Data from the first 10 mins and last 5 mins were excluded from analysis.

The vascular unloading technique uses a plethysmographic device which operates on the finger due to ease of application. These devices in themselves can only measure blood volume changes (not pressure) through the absorption of infrared light. Using the vascular unloading technique, these signals can be transformed into continuous blood pressure. During blood volume changes, an outside counter pressure is continuously exerted to maintain arterial blood volume. These external pressure changes directly correspond to arterial pressure.

5.3.8 Resting and exercise substrate utilisation

During this same resting period, expired gases were measured for 20 mins using a Hans Rudolf breathing mask and analysed online for VO_2 , VCO_2 and ventilation (Cortex metalyser 3B, Leipzig, Germany). Data from the first 10 mins and last 5 mins were excluded from analysis. This enabled us to predict

resting metabolic rate using the Weir equation (Weir 1948) and resting substrate utilisation using RER ($RER = VCO_2/VO_2$).

5.3.9 Intervention

The HIIT group performed 36 cycle ergometry sessions over 12 weeks (3 sessions per week on non-consecutive days) at a local gym. Patients were required to perform at least 32 sessions (89%) for inclusion in analysis. Intensity was based on the 6-20 point Borg RPE scale (Borg 1982) (see Appendix E for Borg scale). The session protocol is outlined in Figure 40. Each session included a 5 min warm up in which participants would progress from RPE 9-13 (“very light to “somewhat hard”) followed by 5 intervals, each with a pedal cadence of >80 RPM reaching RPE 16-17 (“very hard”). The final interval was then followed by a 3 min recovery cycle. HIIT is well documented to be highly effective at improving cardiorespiratory fitness so we had to account for progression throughout the programme. Intervals lasted 2 mins in week 1 and progressed by 10 seconds each week so that week 12 consisted of 3 min 50 second intervals. Three min recovery periods interspersed each interval which consisted of; 90 seconds passive recovery, 60 seconds of band resisted upper body exercise and 30 seconds to prepare for the subsequent interval. The arm resistance bands (Bodymax fitness, Clydebank, UK) were used as a light recovery and involved one exercise per recovery period in the following order: face-pull, horizontal push, horizontal pull, and 30° push (See Appendix E for arm exercises). The initial session was supervised and thereafter participants were guided through each session by voice recorded instructions using an iPod shuffle (Apple Inc. CA, USA). An exercise diary was completed to monitor exercise adherence (See Appendix E for exercise diary). Patients were given an instruction sheet which summarised the intervention instructions (see appendix E).

Apart from HIIT sessions, all study participants were instructed to continue their normal routine and care for 12 weeks and not to change medication, habitual physical activity, diet or body weight. Weekly phone calls were made to assess adherence and habitual physical activity was assessed over 7 days pre and post intervention using a validated multisensory armband (St-onge et al. 2007) (Sensewear; Bodymedia, Pittsburgh, PA).

Figure 40 HIIT protocol



* Interval lengthened by 10 seconds each weeks

Physical Activity

Patients wore a SenseWear (Bodymedia Inc, Pennsylvania, USA) armband on the upper right arm (at the mid-humerus point on the triceps) for seven days (Figure 41). Removal of armband was necessary only for bathing/showering purposes.

The SenseWear armband uses a biaxial accelerometer, heat flux sensor, a galvanic skin response sensor, and a near-body ambient temperature sensor to capture data. Patients age, height, weight, sex, smoking status and hand dominance are inputted so that total energy expenditure can be calculated. Other data is produced as units per day; active energy expenditure, average METs; sedentary time (≤ 2.9 METs); duration of physical activity (> 3.0 METs); duration of moderate physical activity (3.0-5.9 METs); duration of vigorous activity (6.0- 9.0 METs); duration of very vigorous activity (≥ 9.0 METs); number of steps; sleep duration; and duration armband worn.

Figure 41 Habitual physical activity was measured objectively using a validated multisensory armband



5.3.10 Statistics

As this was the first study to examine the impact of HIIT on cardiac structure and function, we could not base our power calculation on our primary outcome. Power was therefore based upon change in HbA_{1c}. We selected a sample size of 12 to provide a statistical power of 80% to detect a difference of 0.6% in HbA_{1c} (Thomas et al. 2006). A sample size 14 was used to allow for 2 dropouts per group. A per-protocol analysis was adopted, as the intention of this study was to assess efficacy and mechanisms of change, not effectiveness. All analyses were performed using SPSS version 19 (IBM, NY, US) and data are presented as means \pm SD, unless otherwise stated.

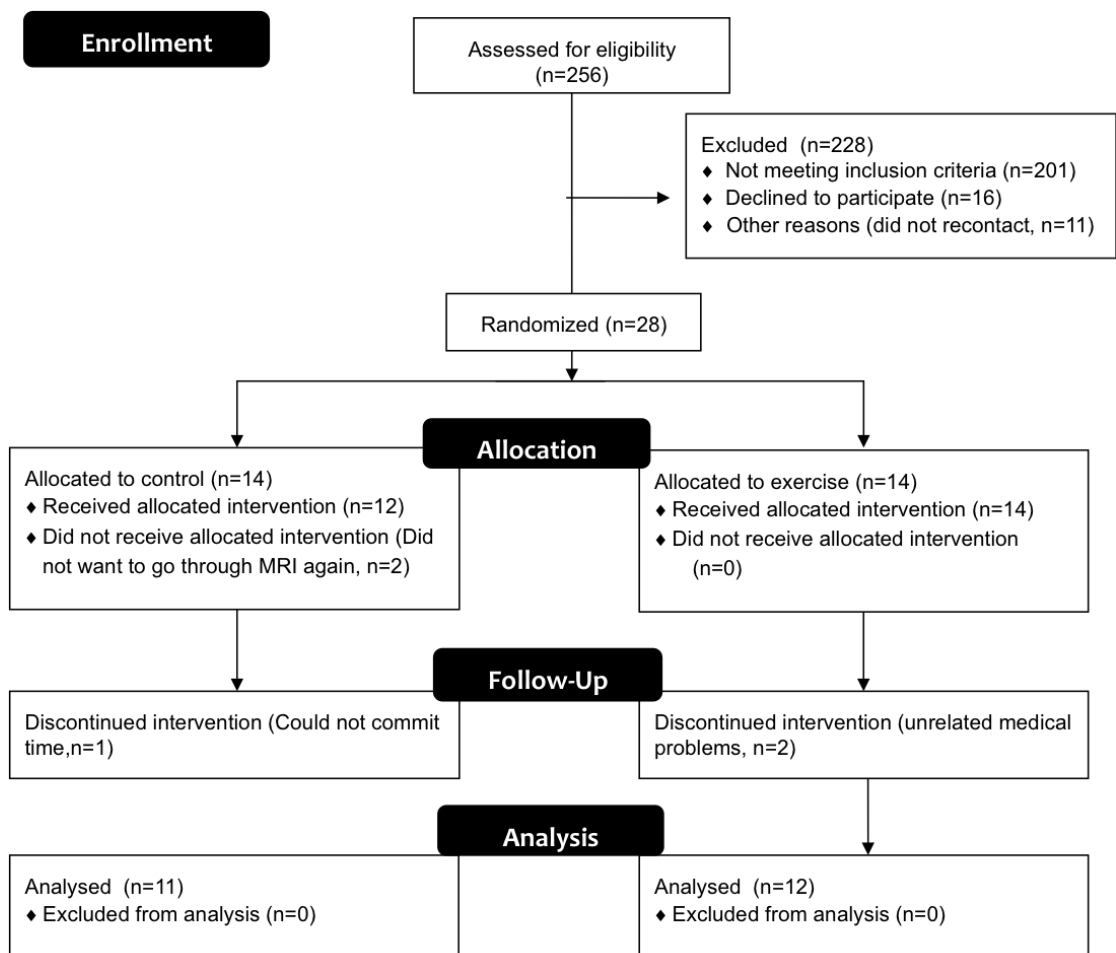
Continuous data were tested for normality using the Shapiro-Wilk test. Comparisons of key baseline variables were made using independent sample t-tests. Between-group comparisons were made using ANCOVA with the baseline value as the covariate. Within group changes were assessed by paired-sample t-test or the non-parametric alternative (Wilcoxon signed rank test) for non-normally distributed data. Adjustment for multiple comparisons was not made due to co-linearity between variables, hypothesis driven comparisons and the increased risk of Type II error following adjustment (Rothman 1990). Pearson's correlation or the non-parametric alternative (Spearman's rank) was used to calculate correlation coefficients between body composition, metabolic and cardiac parameters. P values <0.05 were considered statistically significant.

5.4 Results

5.4.1 Participants

256 individuals were screened for participation in this study. 201 did not meet the inclusion criteria, 16 declined to participate and 11 were lost during re-contact. The resulting 28 adults with Type 2 diabetes (19 of which included in previous chapter) were then randomised into a HIIT (n=14) or control (n=14) group. During the study, two participants left for non-related medical reasons, 1 participant could not commit time and two failed to comply with MRI procedures leaving 12 in the HIIT and 11 in the control group (see Consort diagram Figure 42).

Figure 42 Consort participant flow diagram



Both groups were matched well for all baseline characteristics Table 7. Glycaemic control was similar between groups and liver fat was above the clinically defined threshold for non-alcoholic fatty liver disease (>5% of

hepatocytes are steatotic (Dyson et al. 2014)) in both groups. Adherence to intervention was good, with HIIT patients completing an average of 36 ± 0.9 sessions and Sensewear armband activity revealed no within group change in habitual physical activity (Daily Energy Expenditure: HIIT- 2701 ± 299 to 2537 ± 386 , $p=0.129$ vs. Control- 2548 ± 366 to 2455 ± 166 , $p=0.459$ (calories)).

Table 7 Participant characteristics

Parameter	Control	HIIT	p value
Number (males/females)	8/3	10/2	
Age (years)	59 ± 9	61 ± 9	0.70
Time since diagnosis (years)	4 ± 2	5 ± 3	
BMI (kg/m^2)	32 ± 6	31 ± 5	0.71
Height (cm)	169 ± 9	171 ± 8	0.71
Weight (kg)	90 ± 9	90 ± 15	0.95
HbA _{1c} (%)/ (mmol/mol)	7 ± 0.5 (55 ± 6)	7 ± 1 (54 ± 11)	0.87 (0.88)
Fasting glucose (mmol/l)	7.0 ± 1.0	6.8 ± 1.6	0.693
2-h glucose (mmol/l)	11.7 ± 3.1	12.5 ± 3.1	0.57
Liver fat (%)	7.1 ± 6.8	6.9 ± 6.9	0.94
VO _{2peak} (ml/kg/min)	20.3 ± 6.1	21.8 ± 5.4	0.54
Medications			
Metformin	7	7	
Statins	6	7	
Blood pressure	5	3	

5.4.2 Cardiac structure, function and energetics

HIIT induced structural changes, with a 12% relative increase in left ventricular wall mass ($p<0.05$) and increase in end-diastolic blood volume ($p<0.01$) (Figure 43a). The exercise group also demonstrated improvements in systolic function indicated by raised stroke volume ($p<0.01$) and left ventricular ejection fraction ($p<0.05$). Early diastolic filling rate increased by 24% (Figure 43b) and within group comparison revealed a significant

increase in early filing percentage after HIIT (57 ± 9 to $60 \pm 9\%$, $p < 0.05$; Table 8). There was a 15% relative decrease in peak torsion after exercise (8.1 ± 1.8 to 6.9 ± 1.6 vs. 7.1 ± 2.2 to $7.6 \pm 1.9^\circ$; $p < 0.05$; Figure 43c) and myocardial strain remained constant. The PCr/ATP ratio did not change following HIIT ($p = 0.115$, Table 8).

Table 8 The effect of HIIT on cardiac structure, function and metabolism

	Control			HIIT			Adjusted between group p value _b
	Pre	Post	Within group p value _a	Pre	Post	Within group p value _a	
Cardiac structure							
Left ventricular wall mass (g)	107 ± 25	105 ± 25	0.54	104 ± 17	116 ± 20	0.02*	0.03†
Wall thickness systole (mm)	5.5 ± 1.1	6.2 ± 1.0	0.01**	6.2 ± 1.5	6.8 ± 1.1	0.07	0.54
Wall thickness diastole (mm)	9.1 ± 2.5	10.1 ± 2.5	0.02*	10.7 ± 3.1	11.5 ± 1.8	0.32	0.43
Eccentricity ratio (g/ml)	0.85 ± 0.24	0.87 ± 0.18	0.66	0.94 ± 0.28	0.96 ± 0.24	0.70	0.66
End diastolic volume (ml)	129 ± 28	122 ± 28	0.08	118 ± 30	126 ± 30	0.01**	0.00††
End systolic volume (ml)	50 ± 22	47 ± 22	0.33	42 ± 17	39 ± 13	0.25	0.76
Systolic function							
Systolic blood pressure (mmHg)	126 ± 3	124 ± 5	0.62	123 ± 4	122 ± 4	0.66	0.99
Diastolic blood pressure (mmHg)	84 ± 2	80 ± 2	0.07	81 ± 2	80 ± 2	0.81	0.41
Heart rate (bpm)	63 ± 7	69 ± 13	0.21	67 ± 12	66 ± 16	0.69	0.27
Stroke volume (ml)	79 ± 14	75 ± 15	0.16	76 ± 16	87 ± 19	0.00**	0.00††
Cardiac output (l /min)	5.0 ± 1.0	5.2 ± 1.0	0.54	5.0 ± 1.00	5.5 ± 1.0	0.07	0.31

Ejection fraction (%)	64 ± 11	63 ± 10	0.62	65 ± 8	70 ± 6	0.02*	0.03†
Longitudinal shortening (%)	13.1 ± 2.2	12.7 ± 2.6	0.62	12.2 ± 3.0	13.4 ± 1.8	0.28	0.39
Diastolic function _a							
Early filling percentage (%)	58 ± 11	59 ± 8	0.88	57 ± 9	60 ± 9	0.04*	0.45
Early diastolic filling rate (ml/s)	250 ± 44	251 ± 47	0.68	241 ± 84	299 ± 89	0.01**	0.02†
Late diastolic filling rate (ml/s)	310 ± 143	285 ± 60	0.68	278 ± 67	289 ± 64	0.53	0.56
Strain and torsion _b							
Peak endocardial circumferential strain (%)	23.1 ± 4.1	23.4 ± 4.3	0.82	25.2 ± 4.6	24.5 ± 5.1	0.61	0.82
Peak whole wall circumferential strain (%)	16.5 ± 3.1	16.0 ± 3.3	0.46	16.5 ± 3.1	16.4 ± 4.0	0.94	0.73
Peak torsion (°)	7.1 ± 2.2	7.6 ± 1.9	0.19	8.1 ± 1.8	6.9 ± 1.6	0.04*	0.04†
Metabolism _c							
PCr/ATP ratio	1.76 ± 0.51	1.72 ± 0.36	0.80	1.74 ± 0.39	2.00 ± 0.36	0.19	0.12

Values are mean ± SD. _a Paired t-test. _b Adjusted for baseline value for ANCOVA.

*Significant difference baseline vs. post treatment (p<0.05)

**Significant difference baseline vs. post treatment (p<0.01)

†Significant difference between group interaction (p<0.05)

††Significant difference between group interaction (p<0.01)

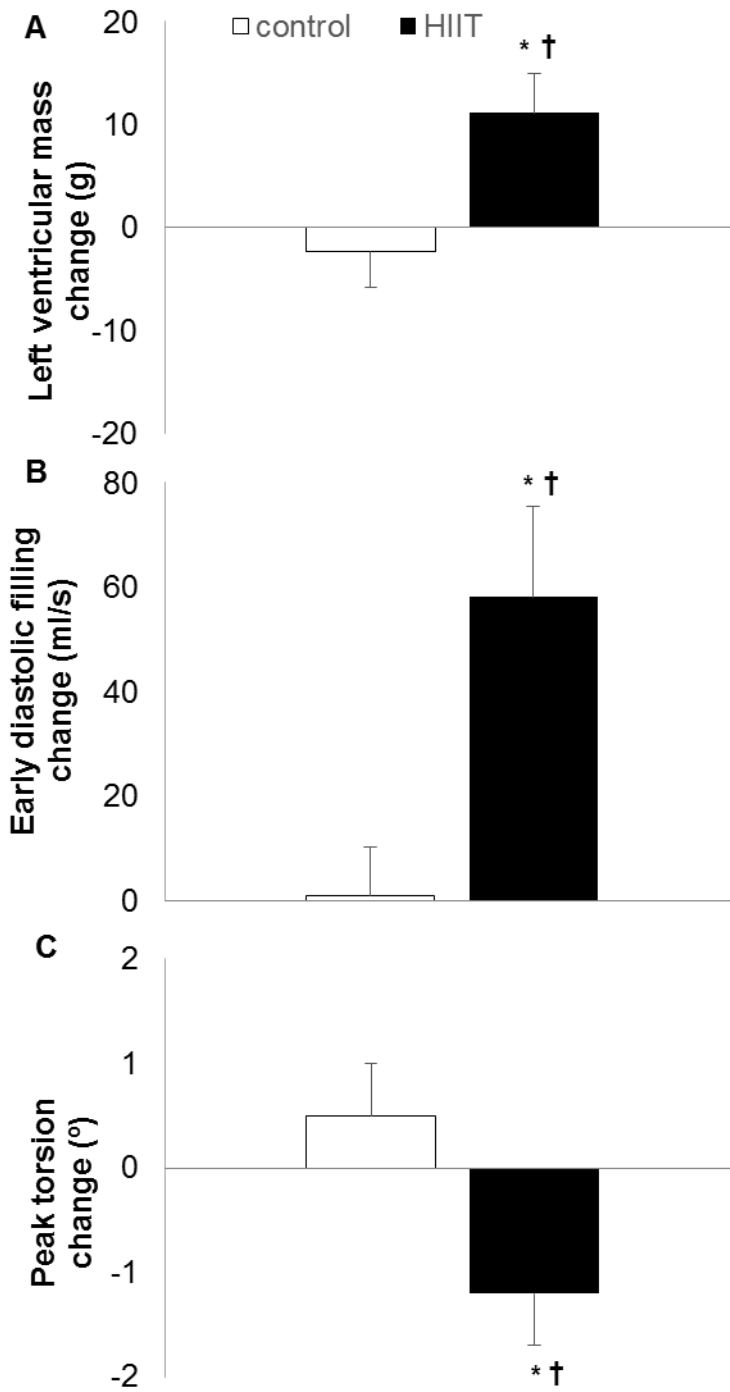
bpm, beats per minute; PCr/ATP, phosphocreatine/adenosine triphosphate.

a n=19. 4 patients not analysed due to abnormal diastolic patterns with no clear diastase (poor images).

b n=19. 4 patients not analysed due to artefact.

c n=18. 5 patients not analysed as large amount of fatty tissue meant the cardiac coil was too far away from chest wall for transmission of signal.

Figure 43 Effect of HIIT vs. control on (a) left ventricular mass, (b) diastolic filling rate and (c) peak torsion. White bars=control, Black bars=HIIT.



Values are means \pm SE.

*Significant difference baseline vs. post treatment ($p < 0.05$)

†Significant difference between group interaction ($p < 0.05$).

5.4.3 Glycaemic control

HIIT had no impact on fasting glucose (6.8 ± 1.6 to 6.8 ± 1.6 mmol/l, $p = 0.866$) or fasting insulin (65.5 ± 39.5 to 65.5 ± 32.8 pmol/l, $p = 0.875$), however, between group comparisons revealed improvements in HbA_{1c}, 2 hour

glucose and glucose AUC ($p < 0.05$; Table 9). There was no improvement in insulin sensitivity (HOMA2-IR and HOMA2-S) or β -cell function (HOMA2- β).

5.4.4 Body composition

Within group comparisons revealed no change in body weight after exercise however the 1% increase and decrease in control and HIIT respectively, was a significant between group interaction ($p < 0.05$; Table 9). There was no effect of HIIT on whole body fat mass but within group comparison revealed a reduction in visceral adipose tissue (201 ± 80 to $181 \pm 72 \text{ cm}^2$, $p < 0.05$; Table 9). Change in whole body fat mass (kg) was associated with 2-h glucose change ($r = 0.46$, $p = 0.027$) and HbA_{1c} change ($r = 0.60$, $p = 0.003$).

5.4.5 Liver fat and enzymes

HIIT elicited a 39% relative reduction in liver fat (6.9 ± 6.5 to $4.2 \pm 3.6\%$, $p < 0.05$) so that 4 patients in the exercise group moved from having clinically significant liver fat to within 'normal' limits ($< 5\%$, Table 9). There was a significant between group interaction for HIIT and liver fat ($P < 0.05$; Table 9), accompanied by within group changes in ALT and AST ($p < 0.5$; Table 9), markers of liver damage. There was large individual variation in liver fat change following HIIT (Figure 44). Change in liver fat across both groups correlated with change in fasting glucose ($r = 0.45$, $p = 0.030$), 2-h glucose ($r = 0.57$, $p = 0.004$) and HbA_{1c} ($r = 0.70$, $p = 0.000$).

Figure 44 Individual liver fat change after 12 weeks of HIIT (red) or control (blue)

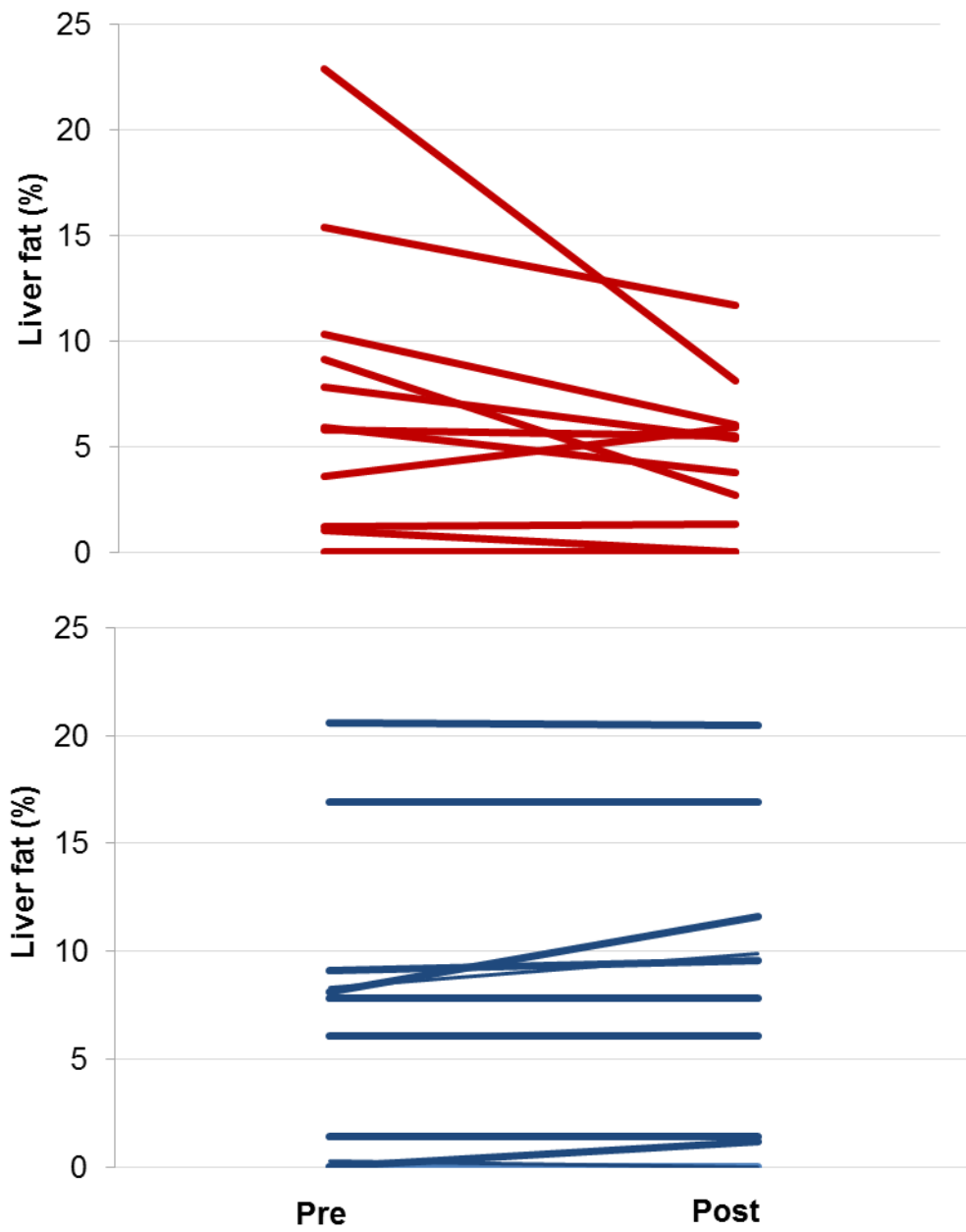


Table 9 The effect of HIIT on body composition, blood parameters and metabolic control

	Control			HIIT			<i>Adjusted between group p value_b</i>
	Pre	Post	<i>Within group p value_a</i>	Pre	Post	<i>Within group p value_a</i>	
Body composition							
Weight (kg)	90 ± 9	91 ± 10	0.06	90 ± 15	89 ± 15	0.09	0.02†
Fat mass (kg)	35.6 ± 10.9	36.0 ± 11.3	0.36	31.9 ± 9.3	30.8 ± 10.2	0.09	0.08
Fat free mass (kg)	54.3 ± 5.9	54.7 ± 5.7	0.28	57.7 ± 9.0	58.2 ± 8.9	0.34	0.72
Visceral adipose tissue (cm ²)	159 ± 58	156 ± 49	0.21	201 ± 80	181 ± 72	0.04*	0.08
Liver fat (%)	7.1 ± 6.8	7.7 ± 6.9	0.12	6.9 ± 6.9	4.2 ± 3.6	0.06	0.01††
Blood parameters							
ALT (U/ l)	34 ± 16	33 ± 14	0.82	36 ± 11	30 ± 10	0.02*	0.14
AST (U/ l)	27.6 ± 10.4	26.5 ± 8.8	0.63	27 ± 7	24 ± 6	0.02*	0.25
ALP (U/ l)	59.2 ± 16.8	61.2 ± 17.5	0.09	66 ± 17	63 ± 16	0.10	0.03†
Total cholesterol (mmol/ l)	4.5 ± 0.9	4.6 ± 0.9	0.62	4.0 ± 1.0	4.5 ± 1.1	0.15	0.77
Triacylglycerol (mmol/ l)	1.1 ± 0.4	1.2 ± 0.4	0.12	1.1 ± 0.3	1.2 ± 0.4	0.28	0.87
Metabolic control							

HbA _{1c} (%) (mmol/mol)	7.2 ± 0.5 (54.9 ± 5.9)	7.4 ± 0.7 (57.0 ± 7.5)	0.07 (0.06)	7.1 ± 1.0 (54.5 ± 10.6)	6.8 ± 0.9 (51.3 ± 10.2)	0.10 (0.09)	0.02†
Fasting glucose (mmol/l)	7.0 ± 1.0	7.6 ± 1.4	0.03*	6.8 ± 1.6	6.8 ± 1.6	0.87	0.15
Fasting insulin (pmol/l)	81.5 ± 46.4	88 ± 39.5	0.42	65.5 ± 39.5	65.5 ± 32.8	0.88	0.22
2-hour glucose (mmol/l)	11.7 ± 3.1	12.9 ± 2.7	0.01**	12.5 ± 3.1	11.7 ± 3.1	0.22	0.02†
2-hour AUGC	1366 ± 66	1544 ± 86	0.01**	1395 ± 81	1399 ± 87	0.94	0.02†
HOMA-IR	1.6 ± 0.9	1.8 ± 0.8	0.40	1.3 ± 0.8	1.4 ± 0.6	0.94	0.19
HOMA2-β	67.8 ± 31.4	67.0 ± 37.3	0.79	68.9 ± 48.6	70.9 ± 49.0	1.00	0.76
HOMA2-S	76.1 ± 33.0	67.3 ± 29.5	0.25	101.7 ± 48.1	98.2 ± 53.8	0.88	0.39

Values are mean ± SD. ^a Paired t-test. ^b Adjusted for baseline value for ANCOVA.

*Significant difference baseline vs. post treatment (p<0.05)

**Significant difference baseline vs. post treatment (p<0.01)

†Significant difference between group interaction (p<0.05)

††Significant difference between group interaction (p<0.01)

5.5 Discussion

This is the first study to examine the effects of HIIT on cardiac structure and function, regional fat deposition and glycaemic control in people with Type 2 diabetes. The main findings were that a 12 week HIIT programme; 1) increased left ventricular wall mass and end-diastolic blood volume, 2) improved systolic and diastolic function, 3) reduced peak torsion and 4) decreased liver fat. HIIT is an effective strategy to reverse cardiac dysfunction and reduce liver fat in this patient group and was accompanied by modest improvements in glycaemic control.

5.5.1 Cardiac changes

Left ventricular wall mass and end-diastolic blood volume increased after 12 weeks of HIIT. This 'physiological hypertrophy' is a known effect of exercise but should not be confused with 'pathological hypertrophy', seen in those with Type 2 diabetes (Frey et al. 2004; Dawson et al. 2005). An increase in cardiomyocyte size and protein synthesis is observed during physiological and pathological hypertrophy, in response to either growth or stress signals respectively (Frey et al. 2004). They differ in that only pathological hypertrophy is characterised by collagen accumulation and increased wall thickness which compromises end-diastolic blood volume and is an independent predictor of cardiovascular death (Liao et al. 1995).

Physiological hypertrophy with a concomitant increase in end diastolic blood volume following exercise in Type 2 diabetes has been observed previously (Schmidt et al. 2013). However, the present study is the first to show that HIIT can stimulate positive cardiac remodelling. It has been previously demonstrated that the magnitude of cardiomyocyte hypertrophy depends on exercise intensity, with higher intensity exercise initiating a larger hypertrophic response (Kemi et al. 2005). These structural adaptations, led to improvements in cardiac systolic function.

The increase in stroke volume and ejection fraction is important because those with Type 2 diabetes have reduced cardiac contractile capabilities (Dawson et al. 2005). Cardiomyocyte responses to high intensity exercise training in animal models have demonstrated improvements in the maximal

extent of shortening as well as contraction and relaxation rates, with twice the improvement seen after training at 85-90% compared to training at 65-70% VO_{2peak} (Kemi et al. 2005). The increased myofilament sensitivity to calcium and the faster rise and diastolic decay of the calcium transient underpins the aforementioned changes (Kemi et al. 2005).

Early filling rate increased by 24% suggesting the myocardium is more compliant and quicker to relax following HIIT. Considering diastolic dysfunction is the most widely reported malfunction of the diabetic heart and an independent predictor of mortality (Rijzewijk et al. 2009; Halley et al. 2011; Diamant et al. 2003), the clinical relevance of these findings are emphasised. One study using echocardiography demonstrated diastolic improvements following 12 weeks of HIIT (Hollekim-Strand et al. 2014), but a longer term intervention using moderate intensity exercise demonstrated no improvements (Hordern et al. 2009). That being said, subgroup analysis revealed improvements when exercise was performed in the vigorous zone (Hordern et al. 2009), highlighting the importance of exercise intensity.

The decrease in torsion after HIIT, is for the first time, evidence that exercise can be used to reverse the raised cardiac torsion observed in Type 2 diabetes (Fonseca et al. 2004) Cardiac torsion is a normal feature of contraction in a healthy heart and reflects the dominance of epicardial fibres over endocardial fibres (Lumens et al. 2006). Raised torsion in Type 2 diabetes is a consequence of impaired contraction of endocardial fibres which are less able to counteract this twisting motion (Lumens et al. 2006). These results indicate that endocardial damage and potential perfusion deficits at the endocardium can be improved with HIIT. Exercise is known to raise cardiac perfusion (Tomanek 1994) but further work is needed to identify the mechanisms which underpin these adaptations. No change was observed in peak endocardial and peak whole wall circumferential strain as the relative contribution of fibres across the myocardial wall remained constant, reflected by the maintained eccentricity ratio.

HIIT stimulated improvements in cardiac structure and function independent of changes in cardiac metabolism. It has been previously suggested that

defects in cardiac metabolism underlie cardiac abnormalities seen in Type 2 diabetes (Diamant et al. 2003) however the decrease in PCr/ATP ratio in Type 2 diabetes most likely reflects changes in substrate supply to the heart rather than an underlying metabolic defect in the myocardium (Cassidy et al. 2015).

5.5.2 Metabolic changes

For the first time, these data reveal that HIIT can reduce liver and visceral fat in Type 2 diabetes which is clinically important because both fat depots play a key pathogenic role in this chronic disease (Björntorp 1991; Taylor 2013). To our knowledge, this is the greatest reduction in liver fat to be reported following exercise in Type 2 diabetes.

Despite this, fasting blood glucose did not change, and in line with healthy adults (Babraj et al. 2009), and obese women (Gillen et al. 2013), the results demonstrate no impact of HIIT on central insulin sensitivity in adults with Type 2 diabetes. These results differ from the very low calorie diet (600 kcal) which led to a 30% relative reduction in liver fat, and normalisation of fasting blood glucose after just 7 days (Lim et al. 2011). In the present study however there was large individual variation in liver fat changes after HIIT, which may explain the absence of change in fasting blood glucose within this small sample. Indeed, those who lost the greatest liver fat had the largest reductions in fasting glucose, reflected in the significant correlation.

It has been reported that HIIT acutely improves peripheral insulin sensitivity when measured within 72 hours of the last exercise bout, attributable to rapid glycogen breakdown and subsequent re-synthesis (Babraj et al. 2009). The two studies which have measured postprandial response to HIIT in Type 2 diabetes, used continuous blood glucose measurements under standard dietary conditions (Little et al. 2011; Gillen et al. 2012). Dietary intake was not standardised in the present study which may explain the lack of within group improvement in 2-hour glucose or HOMA-IR, despite measurements taking place within 48-72 hours of the final exercise session.

Within group analysis also revealed no significant impact of HIIT on HbA_{1c}. The significant between group interactions most likely reflect a worsening of

glycaemic control in the control group. HbA_{1c} reduced by around 0.3% following HIIT, which is less than the reported mean effect (0.6% reduction) of exercise interventions (Thomas et al. 2006). That being said, the greatest improvements in 2-hour glucose and HbA_{1c} occurred in those who lost the largest amount of whole body fat mass and liver fat. Like fasting blood glucose, we speculate that 2-hour and HbA_{1c} group changes failed to reach significance because of the variation in liver fat reductions following HIIT.

This study questions the impact of HIIT on glycaemic control in adults with Type 2 diabetes but also corroborates the importance of ectopic fat in the etiological process of Type 2 diabetes. Patients were required to maintain their weight during the HIIT programme, as we wanted to investigate the effects of exercise without weight loss. Weight loss in Type 2 diabetes has a range of benefits from glucose control to prognosis (Lean et al. 1990), and asking patients to maintain their weight which may have compromised any improvements in glucose control. Interventions to target weight loss and ectopic fat, may be most beneficial for glycaemic improvements. Despite this, these data highlight the positive impact of exercise upon cardiac health, which may be expended further when accompanied by weight loss.

This study is not without limitation. The physiological mechanisms underlying the cardiac adaptations could not be elucidated with the MRI techniques adopted. Myocardial steatosis and perfusion would have provided further insight but due to the duration of MRI scans and tolerability by patients, these techniques could not be used in the present study. Some patients were taking metformin and evidence suggests that metformin may attenuate the effects of exercise through reduced activation of the AMP-activated protein kinase (Sharoff et al. 2010). Using RPE as a guide for exercise intensity rather than an objective measure like heart rate may have limited the accuracy of the training intensity. However, unlike continuous exercise, acute physiological responses to HIIT intervals are *not* predictable and a steady state is *not* achieved (Tschakert et al. 2015). We have found that HR rises incrementally with interval progression e.g. most of our patients did not achieve 90% maximum heart rate until the third interval, but then came close to 100% maximum heart rate at the later end of the third and fourth intervals.

In lights of this, using heart rate as a guide would not have been accurate. RPE has been found to be an accurate predictor of exercise intensity in diabetes (Colberg et al. 2003) and older adults (Shigematsu et al. 2004) and as the exercise programme was designed to be translational, we believed RPE to be more applicable as it could be used by patients to gauge their activities following the study. Finally, dietary monitoring was not adopted throughout the intervention. Self-report food intake is inaccurate in obese individuals (Macdiarmid & Blundell 1998) and there are no food logs validated to provide sensitivity to change over a short 12 week period.

5.6 Conclusions

In summary, this study demonstrates, for the first time, improvement in cardiac structure and function in patients with Type 2 diabetes following a HIIT programme. These changes were accompanied by modest improvements in glycaemic control. HIIT elicited the greatest reduction in liver fat to be recorded following an exercise intervention in Type 2 diabetes and shows that this type of exercise is effective at targeting fat depots which play a role in the aetiology of this chronic condition. The direct benefits of HIIT to glycaemic control remain uncertain, however, HIIT holds potential as a therapy to moderate cardiac risk and reduce liver fat in Type 2 diabetes and should be considered for clinical care alongside other regimens to improve glycaemic control.

Chapter 6 General Discussion

6.1 Key findings

The aim of this thesis was to explore lifestyle related behaviours in cardio-metabolic disease, with a view to improving clinical care. In *chapter 2* we demonstrated that those with cardio-metabolic disease display a cluster of unhealthy lifestyle behaviours. *Chapter 4* highlighted the significant cardiac burden in those with metabolic disease who display no overt cardiac disease. Finally, *chapter 5* provided novel evidence that targeting one of these lifestyle behaviours (exercise) is an effective strategy to moderate cardiac risk in those with metabolic disease. How these findings may impact clinical care will be discussed.

6.2 Implications for clinical care

Data from *chapter 2* indicates that people with cardio-metabolic disease, as a whole, behave differently compared to people without disease. They perform less physical activity, have higher sedentary behaviour and worse sleep patterns. Interestingly, data from the UK Biobank demonstrates a clustering of these lifestyle behaviours, which highlights the need for us to rethink how we approach these behaviours in clinical care. Historically, interventions have focussed on one behaviour, whether physical activity or diet but we have shown people who have low levels of physical activity, are also more likely to have poor sleep and high levels of sitting. NICE guidelines for cardio-metabolic disease, which inform clinical practice, only briefly mention that sitting time should be reduced, but no specific guidelines are made. Sleep is not mentioned at all.

Diet and physical activity are the cornerstones of lifestyle advice for those with cardio-metabolic disease, indeed the first ever national diabetes prevention programme which will target poor diet and physical inactivity, will begin in 2016 (NHS England 2015). Data from *chapter 2* indicate that adults with Type 2 diabetes seem to be changing their diet and suggests that patients are acting upon, or at least aware of, dietary advice. Three in four Type 2 diabetic adults had changed their diet in the last 5 years, compared to only one in four in those without disease. Additionally, half report never to eat

sugar which was significantly more than those with CVD and disease free individuals. In contrast, the proportion of adults reaching the national physical activity recommendations was reduced in Type 2 diabetes. Only one in four of the high diabetes risk group (CVD) had changed their diet and also had low physical activity levels. These data reinforce the need for national programmes to encourage healthy lifestyles, targeting diet and physical activity.

The Health Survey for England (Health and Social Care Information Centre 2014) demonstrated that a high percentage of UK adults could recall the UK fruit and vegetable guidelines (women: 78%, men: 62%), which was proportionally higher than those who could recall the UK physical activity guidelines (women: 29%, men: 27%). In 2011, the UK government published physical activity guidelines which advise all individuals to perform at least 150 mins of moderate activity or 75 mins of vigorous activity per week (Department of health 2011a). A study of representative clinician practices found that exercise and physical activity were only mentioned to 1/6 patients with cardio-metabolic disease and although this was collected from the US, it suggests that physical activity advice is not being adopted in routine clinical practice (Kraschnewski et al. 2013). One strategy which may help is better education of health care professionals. Indeed, General Practitioners, who in the UK have initial contact with the majority of chronic disease patients, reported that they lack education in non-pharmaceutical methods and are uncertain about using lifestyle advice as a treatment (Persson et al. 2013).

There have also been calls for a change in message (Sparling et al. 2015), the 150 min target may be too large for many individuals and may shift emphasis away from the importance of a 'whole day' approach whereby sedentary behaviour is reduced. We know that health benefits are incurred with any increase above the very lowest level of activity and so any improvement needs to be encouraged (Powell et al. 2011). Our data show that almost half of those with the worst cardio-metabolic disease sit for >3 hours each day watching television and advice for reducing sedentary time should be part of lifestyle advice. Reducing sedentary behaviour can be achieved with simple actions such as moving during commercial television breaks,

getting off public transport early and pacing while on the phone. Although the data from *chapter 2* was cross sectional, and therefore cannot confirm that these unhealthy lifestyle behaviours lead to cardio-metabolic disease, they indicate a specific behavioural phenotype in those with Type 2 diabetes and CVD.

Data from *chapter 5* provides evidence that improving lifestyle by adopting a simple exercise routine, has significant cardio-metabolic benefits. *Chapter 4* presents a strong case that patients with metabolic disease who present with no overt cardiac disease, actually have significant preclinical changes in cardiac structure and function. Not only does this data highlight the importance of targeting the often described 'forgotten and fatal complication of diabetes' (Bell 2003), but it also shows the complex interactions between metabolic organs which do not operate in isolation. Early treatments are therefore needed in these patients but a dearth of evidence exists as to effective strategies to reduce cardiac risk.

We have shown improvements in cardiac structure and function after a HIIT intervention in adults with Type 2 diabetes (*chapter 5*) and this novel evidence indicates a potential treatment strategy to reduce cardio-metabolic risk. One of the most commonly cited barriers to physical activity and exercise is 'lack of time' (Trost et al. 2002), another reason why the 150 min target may not be clinically useful. We are unable to recommend HIIT as a robust strategy for all metabolic patients, as this was an efficacy trial showing mechanisms of change. A larger scale population study would need to be undertaken before this type of exercise could be adopted in routine clinical care. HIIT also raises safety concerns due to its high cardiac demand, however large studies in heart failure patients have proved that there is no additional risk compared to moderate exercise (Rognmo et al. 2012). A 'one size fits all' should not be adopted with exercise prescription, and individuals respond differently, however HIIT may provide another alternative.

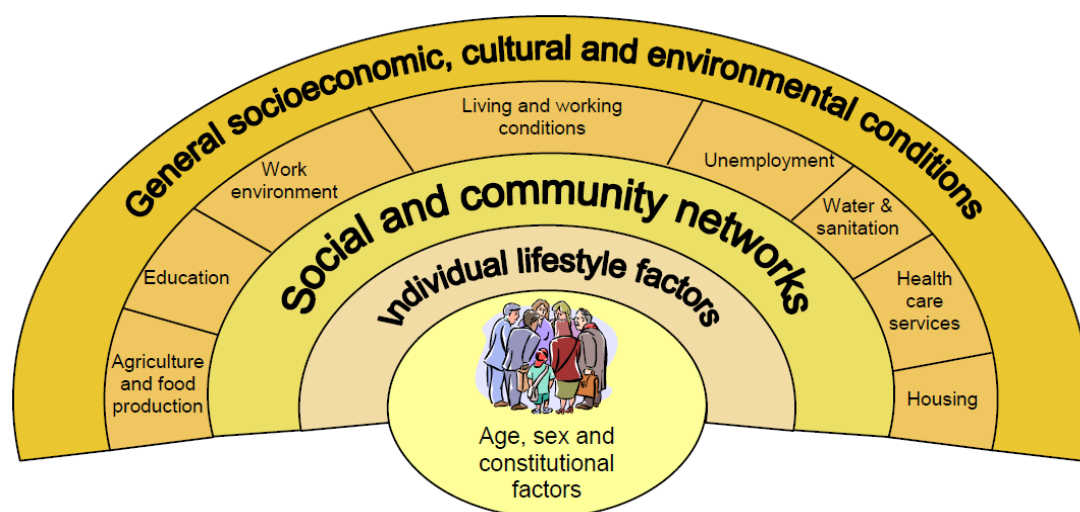
Improvements in glucose control after HIIT, like other exercise interventions, were modest, and suggest that using exercise as a therapy primarily to target glycaemic control, may not be the most effective strategy. The greatest

improvements in glucose control occurred in those who lost the greatest amount of liver and body fat, and interventions to target weight and fat loss may be the most effective strategy for glycaemic improvements. The results also support the hypothesis that liver fat is central to metabolic disturbances (Taylor 2013). That being said, a clear message from this study can be taken; that exercise has significant cardiac benefits and should be promoted in those with metabolic disease. A large proportion of the NHS budget is spent on diabetes complications and we have shown that by adopting a relatively simple exercise regime, we can target one of the largest economic burdens associated with Type 2 diabetes.

Currently, the main pathway to improve physical activity and sedentary behaviour is through 'exercise referral schemes' whereby primary care professional refer patients to third party service providers. However, a recent meta-analysis demonstrated significant uncertainty regarding their effectiveness to increase physical activity and any health related outcomes (Pavey et al. 2011). Change is therefore required. That being said, the responsibility cannot fall solely on healthcare professionals within the clinical setting, rather the government has a large role to play. Data suggests that brief clinical interventions to improve lifestyle behaviours do work in the short term but not in the long term (Campbell et al. 2012). There is a growing belief that we need to move away from a purely behavioural science approach which focuses on individuals, towards a systems approach focusing on populations and complex interactions among physical inactivity correlates (Kohl et al. 2012).

Physical activity and lifestyle needs to become a 'cross-sectoral' priority (MacAuley et al. 2015). Improving lifestyle behaviours will mainly entail changes to public transport, urban infrastructure that creates walk-able spaces, and policies which promote active workplaces and schools. A change in mind-set and cultural shift needs to occur and the government have a large role to play in this.

Figure 45 The social model of health (Dahlgren & Whitehead 1991)



The social model of health by Dahlgren and Whitehead (Dahlgren & Whitehead 1991) (Figure 45), describes the layers of influence on health. There is clearly collective and individual responsibilities for health in which individuals, clinicians and governments all have a role to play. At the centre of the model is an individual with a fixed set of genes who make individual lifestyle choices. The next layer is social and community networks, and the third includes structural factors like working conditions, housing and transport. An individual's health is therefore influenced by a wide range of determinants and if we are going to change the tide, clinical and government strategies need to make sure that all of these determinants enable the 'healthy option to be the easy option'. The world health organisation have recommended multilevel community wide interventions and environments which facilitate healthy lifestyles, rather than just focusing on individualised policy (World Health Organisation 2014).

6.3 Future directions

Although data within this thesis highlight the importance of lifestyle for cardio-metabolic health, a number of questions remain unanswered and provide a platform for future work.

Lifestyle behaviour strategies need improving for those with cardio-metabolic disease. How best a healthy lifestyle can be encouraged and adopted within our society is a pressing question. Technological developments will continue

and using these as a positive re-enforcer of healthy behaviours is likely to be powerful. Current pathways to improve physical activity and lifestyle in clinical care are not effective, therefore designing and developing new pathways for those with cardio-metabolic disease are needed.

Individual responses to physical activity/exercise interventions varies, indeed after the 12 weeks HIIT programme, liver fat changes were between +3% and -10%. Individualised, tailored lifestyle advice may therefore be warranted. Advances in epigenetics, genomics, metabolomics and proteomics in the next 50 years (Booth & Hawley 2015) will allow interdisciplinary research to identify unique predictors of individual susceptibility to metabolic disease and factors which dictate an individual's responses to lifestyle interventions.

Before HIIT could be considered as a routine therapy in clinical care, larger scale effectiveness studies with intention to treat analysis are needed, which also address safety concerns of this type of exercise. We have shown that exercise alone has significant cardiac benefits but weight/fat loss are needed for improvements to glycaemic control. Effective interventions combining diet and exercise which promote weight loss and maintain weight loss over a long period are therefore warranted.

Finally, the exact pathophysiological mechanisms by which metabolic disease leads to cardiac dysfunction and conversely how exercise improves cardiac structure and function are yet to be elucidated. More knowledge in this area will aid a greater depth of understanding of the interacting pathways between the heart and other metabolic organs.

6.4 Conclusions

Over the past decade there has been a dramatic rise in Type 2 diabetes, which is currently the fastest growing disease in the UK and is associated with elevated cardiac risk, hence the term cardio-metabolic disease. Data from this thesis has identified pre-clinical changes to cardiac structure and function in those with Type 2 diabetes, and the need for early interventions. From a large UK population cohort, we have demonstrated that those with cardio-metabolic disease report low levels of physical activity, high sedentary behaviour and poor sleep, and these unhealthy lifestyle behaviours seem to be clustered. Finally, we have demonstrated that improving lifestyle by adopting a relatively simple exercise routine, patients with Type 2 diabetes can reduce cardiac complications. Lifestyle interventions therefore have the potential to significantly reduce co-morbidities associated with cardio-metabolic disease. Despite this, current strategies are lacking and cross sectoral strategies are needed before healthy lifestyles become the norm in modern society.

Appendix A -UK Biobank (chapter 2 documents)

Food Frequency Questionnaire data

	% within each disease group			
	No Disease (n=103,993)	CVD (n=113,469)	Type 2 diabetes without CVD (n=4074)	Type 2 diabetes + CVD (n=11,574)
Oily fish	103,648	113,059	4,057	11,530
Never	10.6	9.8	12.7	11.1
Less than once a week	35.3	30.2	31.9	30.6
Once a week	38.1	38.8	35.3	37.1
2-4 times a week	15.3	20.1	18.3	20.0
5-6 times a week	0.7	0.8	1.2	0.9
Once of more daily	0.2	0.3	0.6	0.4
Processed meat	103,891	113,330	4,061	11,554
Never	10.2	7.9	8.6	6.4
Less than once a week	30.9	29.6	27.2	25.0
Once a week	29.0	29.7	28.1	29.6
2-4 times a week	26.2	28.7	30.4	33.9
5-6 times a week	3.0	3.3	4.3	4.1
Once of more daily	0.7	0.9	1.4	1.1
Poultry intake	103,916	113,349	4,069	11,551
Never	5.8	4.0	5.1	3.7
Less than once a week	10.4	10.5	10.7	11.2
Once a week	36.0	36.3	34.2	35.3
2-4 times a week	45.6	46.9	46.8	47.1
5-6 times a week	1.9	2.1	2.7	2.3
Once of more daily	0.3	0.3	0.5	0.4
Cheese intake	102,188	110,315	3,900	11,141
Never	2.1	3.1	3.7	4.1
Less than once a week	14.9	18.5	23.2	21.2
Once a week	20.4	22.4	23.1	24.7
2-4 times a week	47.3	44.3	39.8	40.9
5-6 times a week	11.2	8.5	7.6	6.3
Once of more daily	4.0	3.1	2.6	2.8
Salt intake	103,979	113,457	4,073	11,573
Never/Rarely	56.5	58.4	54.3	55.5
Sometimes	28.0	26.3	28.7	28.0

Usually	11.3	10.8	12.0	11.9
Always	4.2	4.5	5.1	4.6
Non-oily fish intake	<i>103,705</i>	<i>113,135</i>	<i>4,055</i>	<i>11,516</i>
Never	4.7	4.0	5.7	4.8
Less than once a week	29.9	27.3	28.9	26.9
Once a week	49.8	51.1	47.9	49.9
2-4 times a week	15.0	16.8	16.4	17.6
5-6 times a week	0.5	0.5	0.8	0.5
Once of more daily	0.1	0.2	0.2	0.3
Beef intake	<i>103,790</i>	<i>113,154</i>	<i>4,057</i>	<i>11,534</i>
Never	11.6	9.3	12.1	9.3
Less than once a week	46.0	45.4	42.7	41.6
Once a week	31.8	33.0	32.6	34.9
2-4 times a week	10.5	12.0	12.2	13.7
5-6 times a week	0.2	0.2	0.4	0.3
Once of more daily	0.1	0.1	0.1	0.1
Lamb/mutton intake	<i>103,671</i>	<i>112,946</i>	<i>4,053</i>	<i>11,514</i>
Never	18.1	16.1	15.5	14.9
Less than once a week	58.1	56.2	52.8	51.8
Once a week	21.1	24.4	26.2	28.4
2-4 times a week	2.6	3.1	5.3	4.7
5-6 times a week	0.1	0.1	0.1	0.1
Once of more daily	0.0	0.0	0.0	0.1
Pork intake	<i>103,671</i>	<i>112,999</i>	<i>4,057</i>	<i>11,516</i>
Never	17.7	15.5	18.4	15.5
Less than once a week	58.4	56.7	51.9	51.8
Once a week	20.8	24.0	24.6	27.5
2-4 times a week	3.0	3.7	4.9	5.0
5-6 times a week	0.1	0.1	0.1	0.2
Once of more daily	0.0	0.0	0.1	0.1
Milk type used	<i>103,940</i>	<i>113,415</i>	<i>4,070</i>	<i>11,571</i>
Full cream	7.9	5.9	5.6	4.9
Semi-skimmed	66.3	64.0	65.6	66.2
Skimmed	18.1	21.9	21.4	22.2
Soya	3.7	3.5	3.5	2.5
Other type of milk	1.0	1.2	0.8	1.1
Never/rarely have milk	3.1	3.5	3.2	3.1
Spread type	<i>103,846</i>	<i>113,315</i>	<i>4,059</i>	<i>11,550</i>
Never/rarely use	11.3	10.7	10.4	8.4

Butter/spreadable	39.5	33.0	28.3	29.9
Flora pro active/benecol	0.4	0.6	0.6	0.7
Other type spread/marg	48.8	55.7	60.7	61.1
Bread type	<i>100,505</i>	<i>109,974</i>	<i>3,973</i>	<i>11,352</i>
White	24.2	27.9	23.8	29.7
Brown	12.6	12.9	14.0	13.0
Wholemeal/wholegrain	59.7	55.3	58.1	53.7
Other	3.5	3.9	4.2	3.6
Cereal type	<i>85,959</i>	<i>92,847</i>	<i>3,479</i>	<i>9,744</i>
Bran	17.1	16.7	16.1	16.9
Biscuit (e.g. Weetabix)	16.8	18.3	23.9	24.6
Oat (porridge)	23.9	26.8	29.2	29.0
Muesli	23.7	18.4	14.7	12.5
other	18.4	19.8	16.2	17.0
Never eat	<i>103,848</i>	<i>113,190</i>	<i>4,039</i>	<i>11,527</i>
Eggs or foods containing eggs	2.1	3.3	4.4	4.6
Dairy products	1.6	2.6	4.1	3.5
Wheat products	1.6	2.9	3.2	3.0
Does your diet vary from week to week?	<i>103,701</i>	<i>113,167</i>	<i>4,052</i>	<i>11,534</i>
Never/rarely	38.0	32.3	29.8	27.6
Sometimes	55.1	58.8	59.9	61.8
Often	6.9	8.9	10.3	10.6
Bread intake (slices of bread each week) <i>mean (SD)</i>	<i>12.1 (8.5)</i>	<i>12.7 (8.7)</i>	<i>14.4 (9.8)</i>	<i>14.6 (9.3)</i>
Cereal intake (bowls of cereal per week) <i>mean (SD)</i>	<i>4.5 (2.8)</i>	<i>4.4 (2.8)</i>	<i>4.8 (2.8)</i>	<i>4.5 (2.8)</i>

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

No vigorous physical activities → **Skip to question 3**

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

No moderate physical activities → **Skip to question 5**

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

No walking → **Skip to question 7**

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

List of diseases included in the CVD group

hypertension
heart/cardiac problem
peripheral vascular disease
venous thromboembolic disease
essential hypertension
gestational hypertension/pre-eclampsia
angina
heart attack/myocardial infarction
heart failure/pulmonary odema
heart arrhythmia
heart valve problem/heart murmur
cardiomyopathy
pericardial problem
stroke
transient ischaemic attack (tia)
subdural haemorrhage/haematoma
subarachnoid haemorrhage
leg claudication/ intermittent claudication
arterial embolism
pulmonary embolism +/- dvt
deep venous thrombosis (dvt)
peripheral neuropathy
ischaemic stroke
mitral valve disease
mitral regurgitation / incompetence
aortic valve disease
aortic regurgitation / incompetence
hypertrophic cardiomyopathy (hcm / hocm)
pericarditis
pericardial effusion
aortic aneurysm rupture
aortic dissection
aortic stenosis
brain haemorrhage

Socio-demographics of those who have missing data on physical activity, sitting or sleep and therefore excluded from analysis (n=60,938).

	% within each disease group			
	No Disease (n=23,515) 20.7% missing	CVD (n=32,928) 18.4% missing	Type 2 diabetes without CVD (n=1104) 22.5% missing	Type 2 diabetes with CVD (n=3391) 21.3% missing
SOCIO-DEMOGRAPHICS				
% Male	39.2	40.6	53.8	53.0
Age (n)	23,515	32,928	1104	3391
37-49	31.1	10.4	13.1	5.5
50-59	36.5	28.9	29.5	26.9
60-73	32.4	60.7	57.3	67.6
BMI (n)	22,903	32,651	1090	3356
<18.5-24.9 (under and acceptable weight)	40.5	20.4	12.6	6.9
25-29.9 (overweight)	42.1	40.7	37.2	29.8
≥30 (obese)	17.4	39.0	50.3	63.4
Townsend deprivation quintile (n)	23,494	32,892	1099	3387
1 (least deprived)	19.4	16.6	15.7	12.1
2	18.9	18.0	15.2	14.1
3	20.0	19.0	18.4	17.6
4	19.9	19.8	18.7	22.0
5 (most deprived)	21.8	26.7	32.0	34.3
Ethnicity (n)	22,828	32,574	1092	3365
White/British	92.5	93.8	83.2	86.9
Mixed	0.7	0.5	0.5	0.7
Asian	2.5	2.0	9.8	6.5
Black African	2.2	2.4	3.8	3.8
Chinese	0.6	0.2	0.3	0.2
Other	1.4	1.0	2.4	1.8

Appendix B -Consent form



Institute of Cellular Medicine
William Leech Building
Newcastle University
Framlington Place
Newcastle upon Tyne
NE2 4HH
T: +44 (0)191 222 5851



Patient Identification number for this trial:

CONSENT FORM

Title of Project: Exercise and non-alcoholic fatty liver disease

TYPE 2 DIABETES PATIENTS

Name of researchers: Dr M Trenell, Dr K Hollingsworth, Professor R Taylor, Professor C Day.

Please initial box

1. I confirm that I have read and understand the information sheet dated (version.....) for the above study and have had the opportunity to ask questions.
2. I understand that my participant is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.
3. I agree to my GP being informed of my participation in the study
4. I agree to take part in the above study.
5. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the NHS Trust, whereit is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Appendix C -Screening documents (PARQ, medical history and physical examination to determine any contraindications for exercise testing)

Visit 1

Physical Activity Readiness Questionnaire

Name: _____

Date of Birth: _____

		Please choose	
1	Has your doctor ever said that you have a <i>heart condition</i> and that you should only do physical activity recommended by a doctor?	YES	NO
2	Do you ever feel <i>pain</i> in your chest when you do physical activity?	YES	NO
3	Have you ever had chest pain when you are not doing physical activity?	YES	NO
4	Do you ever feel <i>faint or have spells of dizziness</i> ?	YES	NO
5	Do you have a <i>joint problem</i> (also back problem) that could be made worse by exercise?	YES	NO
6	Have you ever been told that you have high blood pressure?	YES	NO
7	Do you have any breathing problems?	YES	NO
8	Do you have any problems with your liver, thyroid, kidneys or have diabetes?	YES	NO
7	Are you currently taking any <i>medication</i> ? If so, what? _____ Reason _____	YES	NO
8	Are you pregnant, have you had a baby in the last 6 months, or do you plan to have a baby this year?	YES	NO
9	Has your mother or father had any heart problems?		
10	How many times a week do you do exercise: _____		
11	Is there any other reason why you should not participate in physical activity? If so, what? _____	YES	NO

Signed by (staff): _____ Print: _____

Date: _____

Name: _____
DOB: _____ Age: _____
Study ID: _____
General Practitioner: _____
GP Address: _____

Height _____
Weight _____
Waist _____
Hip circ. _____
Blood Pressure _____
Smoker YES NO or given up <6month
Blood Taken: YES NO
Comment: _____

To be completed by investigator:

Risk Stratification (circle):	Low	Moderate	High
Action Taken: Commence exercise (Low Risk)			YES / NO
	Referred for Exercise ECG (moderate and high risk)		YES / NO

Completed by _____ Date _____

Medical Diagnosis:

History of cardiovascular disease	YES	NO
Peripheral vascular disease	YES	NO
Hypertension	YES	NO
Diabetes	YES	NO
Pulmonary disease	YES	NO

Previous Physical Examination

Have you had anything reported previously from a physical examination?	YES	NO
--	-----	----

History of symptoms

Discomfort in the chest, jaw, neck, back or arms (e.g. pressure, tingling, pain, heaviness, burning, tightness, squeezing or numbness)	YES	NO
--	-----	----

Light headedness, dizziness or faint?	YES	NO
---------------------------------------	-----	----

Recent Illness

Hospitalisation, new medical diagnosis, surgery	YES	NO
---	-----	----

Details _____

Orthopaedic problems

Arthritis, joint swelling, anything which would make exercise difficult	YES	NO
---	-----	----

Medication use

Medication	YES	NO
------------	-----	----

Details _____

Allergies _____

Details _____

Other habits

Caffeine	YES	NO	if yes, units per week	___
Alcohol	YES	NO	if yes, units per week	___
Tobacco	YES	NO	if yes, units per week	___

Exercise history

Frequency (/week)	1	2	3	4	5	6	7	8
Duration per session (min)		10	20	30	40	50	60	70

Work history

Focus on current of expected physical demands

Family history

Cardiac	YES	NO
Pulmonary	YES	NO
Metabolic disease	YES	NO
Stroke	YES	NO
Sudden death	YES	NO

Comments:

Completed by

Date

Appointment 1**Physical examination**

Name: _____ **DOB:** ___/___/___

Body weight (kg): _____ **Waist Circumference (cm):** _____

%Fat Free Mass: _____ **% Fat Mass:** _____

Apical pulse rate(min): _____ **Rhythm:** _____ **OK / Not OK**

Resting blood pressure, seated. ___ / ___

Auscultation of the lungs **OK / Not OK**
with specific attention to uniformity of breath sounds in all areas (absence of rales and wheezes)
 Comment: _____

Palpation of cardiac apical impulse **OK / Not OK**
point of maximal impulse
 Comment: _____

Auscultation of the heart **OK / Not OK**
with specific attention to murmurs, gallops, clicks and rubs.
 Comment: _____

Evaluation of the abdomen **OK / Not OK**
Bowel sounds, masses, visceromegaly, and tenderness.
 Comment: _____

Evaluation of lower extremities **OK / Not OK**
Oedema and presence of arterial pulse.
 Comment: _____

Inspection of the skin **OK / Not OK**
focus on lower extremities in people with diabetes.
 Comment: _____

Neurologic function **OK / Not OK**
Reflexes
 Comment: _____

Any orthopedic or medical condition that would limit exercise. **YES / NO**
 Comment: _____

Ventricular tachycardia **OK / Not OK**
 Comment: _____

ST elevation (+1.0 mm) in leads without diagnostic Q-waves (other than V₁ or aVR) **OK / Not OK**
 Comment: _____

ST or QRS changes such as excessive ST suppression >2mm horizontal or down sloping ST-segment depression **OK / Not OK**
 Comment: _____

Arrhythmias other than: sustained ventricular tachycardia, including multiple PVCs, triplets of PVCs, supraventricular tachycardia, heart block, or bradyarrhythmias. **OK / Not OK**
 Comment: _____

Cleared to start exercise test **YES / NO**

Completed by: _____ **Date** _____

Physical examination (page 2 on reverse of page 1)

Exercise Stress Testing

Exercise Protocol: _____

Absolute indicators for terminating the Exercise Stress test:

Drop in blood pressure of >10mm Hg <i>from baseline blood pressure despite an increase in workload, when accompanied by other evidence of ischemia.</i>	OK / Not OK Comment: _____ _____
Any form of chest pain or shortness of breath	OK / Not OK Comment: _____ _____
Increasing nervous system symptoms (e.g. ataxia, dizziness or near syncope)	OK / Not OK Comment: _____ _____
Technical difficulties monitoring ECG or blood pressure	OK / Not OK Comment: _____ _____
Ventricular tachycardia	OK / Not OK Comment: _____ _____
ST elevation (+1.0 mm) in leads without diagnostic Q-waves (other than V ₁ or aVR)	OK / Not OK Comment: _____ _____
ST or QRS changes <i>such as excessive ST suppression >2mm horizontal or down sloping ST-segment depression</i>	OK / Not OK Comment: _____ _____
Arrhythmias other than: <i>sustained ventricular tachycardia, including multiple PVCs, triplets of PVCs, supraventricular tachycardia, heart block, or bradyarrhythmias.</i>	OK / Not OK Comment: _____ _____
Fatigue, shortness of breath, wheezing, leg cramps, or patient develops discomfort.	OK / Not OK Comment: _____ _____
Development of bundle-branch block or intraventricular conduction delay <i>that cannot be distinguished from ventricular tachycardia</i>	OK / Not OK Comment: _____ _____
Hypertensive response Systolic blood pressure of > 250 mm Hg and / or diastolic pressure of >115 mm Hg	OK / Not OK Comment: _____ _____

Comments:

Adverse reaction to exercise: YES / NO

Cleared to start exercise: YES / NO

Appendix D -MRI screening questionnaire

Volunteer's name: _____ Date of birth ____ / ____ / ____

Weight: _____ Height: _____

Please check the following carefully.

Have you had any surgery:	Yes	No
Have you had any operations/procedures involving your head, chest or heart?		

Do you have any of the following?

Cardiac pacemaker, aneurysm clip, stent, heart valve replacement, cochlear implant, programmable shunt, spinal stimulation wires, or any other implants.	Yes	No
---	------------	-----------

Is there any possibility that you could have metal fragments in your eye?	Yes	No
Do you have any metal fragments anywhere in your body?	Yes	No

Are you wearing?

Dentures with metal	Yes	No
A hearing aid	Yes	No
Body piercing/jewellery/hair grips	Yes	No
Slow-release drug patches on your skin	Yes	No

Do you have any tattoos?	Yes	No
Have you ever had a fit or blackout?	Yes	No
Do you have epilepsy or diabetes?	Yes	No

<i>FOR WOMEN OF CHILDBEARING AGE:</i> Could you be pregnant?	Yes	No
--	------------	-----------

Some items can interfere with MR examinations, and may also be hazardous to your safety

ALL metal worn or carried on your person must be removed

I understand the procedure of a MRI examination. I also understand the above questions.

Volunteer's Signature: _____ Date: _____

Staff Signature: _____ Date: _____

Appendix E -HIIT intervention documents (*chapter 5*)
Borg scale

Rate of Perceived Exertion

6	No exertion at all
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	Very hard
17	Very, very hard
18	
19	
20	

Progression for warmup

Goal for intervals

Arm resistance band exercises

Exercise Picture Guide

Face Pull - Start



Face Pull - Finish



Chest Press - Start



Chest Press - Finish



Row - Start



Row - Finish



High Press - Start



High Press - Finish



Exercise diary

Exercise Diary

Name: _____

Please fill in this diary after every exercise session:

Session	Date	Max Heart Rate	Observations (e.g. recent illness, feeling of fatigue...)
1	/ /		
2	/ /		
3	/ /		Weight (Kg) _____
4	/ /		
5	/ /		
6	/ /		Weight (Kg) _____
7	/ /		
8	/ /		
9	/ /		Weight (Kg) _____
10	/ /		
11	/ /		
12	/ /		Weight (Kg) _____
13	/ /		
14	/ /		
15	/ /		Weight (Kg) _____
16	/ /		
17	/ /		
18	/ /		Weight (Kg) _____

Exercise Diary

Session	Date	Max Heart Rate	Observations (e.g. recent illness, feeling of fatigue...)
19	/ /		
20	/ /		
21	/ /		Weight (Kg) _____
22	/ /		
23	/ /		
24	/ /		Weight (Kg) _____
25	/ /		
26	/ /		
27	/ /		Weight (Kg) _____
28	/ /		
29	/ /		
30	/ /		Weight (Kg) _____
31	/ /		
32	/ /		
33	/ /		Weight (Kg) _____
34	/ /		
35	/ /		
36	/ /		Weight (Kg) _____

HIIT instruction sheet

High Intensity Intermittent Training Study

What you need

- 1 iPod shuffle with 12 exercise guidance tracks;
- 1 Exercise band
- 1 Laminated *Rate of Perceived Exertion* and *Exercise Picture Guide*
- 1 Exercise diary sheet

What to do

Please do **three sessions per week** with the aim of completing 36 sessions in **12 weeks**. Aim for a full day between each session when possible. We recommend against doing more than two sessions on consecutive days.

There are 12 tracks on your iPod. Every week/three sessions you will progress to the next track. So, track one for week one (or sessions 1-3), track two for week 2... track 12 for week 12.

Please record each session on the exercise diary sheet provided.

How hard to exercise

Each session consists of a warmup, five 'intervals' of hard cycling, some light resistance band exercises, and a cool down.

During the intervals, which are 2 minutes long in week one and get slightly longer each week, we would like you to work what you consider *very hard* or 16-17 on the *Rate of Perceived Exertion* scale provided. You should feel out of breath by the end of an interval and be breathing hard throughout.

Adjust the level of resistance on the bike so you can just pedal at a speed of 80-100 revolutions per minute.

What else to do

Please maintain your current diet and lifestyle outside of the exercise time. Weigh yourself weekly, preferably at the same time on the same day of the week. It's important you maintain your starting weight.

For your interest

The muscles that should be doing the bulk of the work when you do the resistance band exercises are in red.

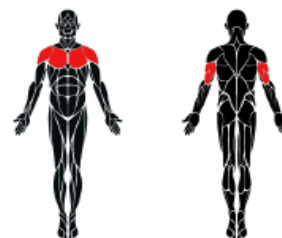
Face-Pull



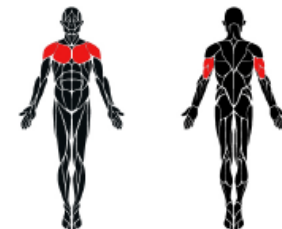
Row



Chest Press



High Press



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