Physical Activity, Exercise and Non-Alcoholic Fatty Liver Disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver conditions ranging from hepatic steatosis through steatohepatitis to cirrhosis. Its prevalence has been estimated at between one-in-five and one-in-three of the adult population depending on country and diagnostic criteria used. Prevalence increases with degree of obesity, and is very common in those with Type 2 diabetes (T2DM). Rising prevalence of obesity and T2DM, particularly in younger people, will ensure that NAFLD remains a growing clinical concern for the future.

Lifestyle modification, which encompasses diet, weight loss, physical activity, and/or exercise related behaviours, is the primary recommended therapy for NAFLD, especially in the absence of approved pharmaceutical agents. Despite lifestyle modifications being central to the management of NAFLD, the evidence base upon which these guidelines are based is lacking, and this is particularly true for physical activity and exercise.

The focus of this thesis is on defining, exploring and developing the evidence for physical activity and exercise in NAFLD with a view to improving clinical care. The work contained within this thesis demonstrates that low levels of physical activity are prominent in people with NAFLD and that targeting this with resistance exercise therapy confers benefits to both liver lipid and the factors promoting its accumulation. It also highlights alterations in cardiac structure and function in people with NAFLD in the absence of overt cardiac disease, which may provide a therapeutic avenue in which to decrease cardiac disease risk in people with fatty liver. Over the duration of the work described in this thesis, the number of studies reporting on exercise and liver fat in people with NAFLD has increased markedly. The new information contained within this thesis contributes to this body of knowledge and, over time, will improve the management of a condition that is an increasing burden to the people of the Western world.

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Publications, Presentations and Awards

Publications

Moore S, **Hallsworth K**, Bluck LJC, Ford GA, Rochester L, Trenell MI. Measuring energy expenditure following stroke: validation of a portable device. *Stroke.* 2012 June;43(6):1660-62.

Jakovljevic DG, Moore S, **Hallsworth K**, Fattakhova G, Thoma C, Trenell MI. Comparison of cardiac output determined by bioimpedance and bioreactance methods at rest and during exercise. *Journal of Clinical Monitoring and Computing*. 2012 April;26(2):63-68.

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Newton JL, Pairman J, **Hallsworth K**, Moore S, Plötz T, Trenell MI. Physical activity intensity but not sedentary activity is reduced in chronic fatigue syndrome and is associated with autonomic regulation. *QJM*. 2011 Aug;104(8):681-7.

Conference Presentations

- **Hallsworth K**, Hollingsworth KG, Thoma C, Taylor R, Day CP, Trenell MI. Cardiac structure and function is altered in adults with non-alcoholic fatty liver disease. EuroPRevent: The European Society of Cardiology Congress 2012, Dublin, Ireland.

- **Hallsworth K**, Hollingsworth KG, Thoma C, Taylor R, Day CP, Trenell MI. Cardiac structure and function is altered in adults with non-alcoholic fatty

liver disease. EASL: The International Liver Congress 2012, Barcelona, Spain.

- **Hallsworth K**, Thoma C, Moore S, Taylor R, Day CP, Trenell MI. Physical activity levels are lower in people with non-alcoholic fatty liver disease than matched healthy controls. Diabetes UK Annual Professional Conference 2012, Glasgow, UK.

- **Hallsworth K**, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, Day CP, Trenell MI. Resistance exercise improves liver fat and glucose control in people with non-alcoholic fatty liver disease. The Chartered Society of Physiotherapy Annual Congress 2011, Liverpool, UK.

- **Hallsworth K**, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, Day CP, Trenell MI. Resistance exercise improves liver fat, fat oxidation and glucose control in adults with non-alcoholic fatty liver disease. EASD: The European Association for the Study of Diabetes Annual Meeting 2011, Lisbon, Portugal.

- **Hallsworth K**, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, Day CP, Trenell MI. Resistance exercise improves liver fat, fat oxidation and glucose control in adults with non-alcoholic fatty liver disease. EASL: The International Liver Congress 2011, Berlin, Germany.

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- **Hallsworth K**, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, Day CP, Trenell MI. Resistance exercise improves liver fat and glucose

control in people with non-alcoholic fatty liver disease. Diabetes UK Annual Professional Conference 2011, London, UK.

- **Hallsworth K**. Exercise and NAFLD. Lifelong Health and Wellbeing Research Retreat, Linden Hall, Northumberland, UK.

Lay Presentations

- "Does Exercise have to be Aerobic?" - Oral presentation to Diabetes UK Benefactors (Newcastle University – chaired by Prof. George Alberti)
- "The Effects of Weight Training in Adults with NAFLD"- Oral presentation at our NAFLD Patient Feedback Event, Newcastle University

Awards

- Best Poster Presentation. Lifelong Health and Wellbeing Research Retreat, Linden Hall, Northumberland , UK
- Young Investigator's Bursary awarded for EASL 2011
- Young Investigator's Bursary awarded for EASL 2012
- Abstract voted in the "Top 10%" at EASL 2012
- Poster awarded "Judge's Choice Award" at EuroPRevent 2012

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List of Abbreviations

Abbreviation	Meaning
α- ARs	α adrenergic receptors
ACSM	American College of Sports Medicine
AEE	active energy expenditure
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AT	anaerobic threshold
ATP	adenosine triphosphate
AUC	area under the curve
β - ARs	β adrenergic receptors
BMI	body mass index
BMR	basal metabolic rate
BP	blood pressure
BSA	body surface area
CHREBP	carbohydrate response element binding protein
CV	cardiovascular
DBP	diastolic blood pressure
DLW	doubly labelled water
DNL	*de novo* lipogenesis
ECG	electrocardiogram
EDV	end diastolic volume
EDVI	end diastolic volume index
EE	energy expenditure
EPOC	excess post-exercise oxygen consumption
ESV	end systolic volume
ESVI	end systolic volume index
fsOGTT	frequently sampled oral glucose tolerance test
GGT	gamma-glutamyltransferase
GLUT	glucose transporter
GP	general practitioner

HbA1c	glycosolated haemoglobin
HDL	high density lipoprotein
HOMA-IR	homeostasis model assessment of IR
HSL	hormone-sensitive lipase
IHL	intrahepatic lipid
IML	intramuscular lipid
IPAQ	International Physical Activity Questionnaire
IR	insulin resistance
LDL	low density lipoprotein
LPL	lipoprotein lipase
LV	left ventricle/ventricular
MET	metabolic equivalent
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NEAT	non-exercise activity thermogenesis
NEFA	non-esterified fatty acids
NEFA-S	NEFA-suppression
NHS	National Health Service
1RM	one repetition maximum
PCr	phosphocreatine
PET	positron emission tomography
PNPLA3	patatin-like phospholipase domain-containing protein 3
RER	respiratory exchange ratio
RQ	respiratory quotient
SAT	subcutaneous adipose tissue
SBP	systolic blood pressure
SPSS	statistical package for the social sciences
SREBP	sterol-regulatory-element binding protein
T2DM	Type 2 diabetes
TEE	total energy expenditure
TNF-α	tumour necrosis factor alpha

TSR	torsion-to-shortening ratio
VAT	visceral adipose tissue
vLDL	very low density lipoprotein
VO_2max	maximal oxygen consumption
W	watts

Chapter 1: Introduction and Literature Review

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Chapter 1: Introduction and Literature Review

1.1 General Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver conditions ranging from hepatic steatosis through steatohepatitis to cirrhosis (Day, 2006). Its prevalence has been estimated at between one-in-five and one-in-three of the adult population depending on country and diagnostic criteria used (Barshop *et al.*, 2008). Prevalence increases with degree of obesity (Harrison and Day, 2007; Fabbrini *et al.*, 2010), and is very common in those with Type 2 diabetes (T2DM) (Kotronen *et al.*, 2008). Rising prevalence of obesity and T2DM, particularly in younger people, will ensure that NAFLD remains a growing clinical concern for the future.

Accumulation of excess liver fat is the first step in the development of NAFLD (Day, 2006). NAFLD has been linked to insulin resistance (IR)(Angelico *et al.*, 2005), and is an independent risk factor for T2DM (Sattar *et al.*, 2007), and cardiovascular disease (Targher *et al.*, 2010). Lifestyle modification, which encompasses diet, weight loss, physical activity, and/or exercise related behaviours, is the primary recommended therapy for NAFLD (Loria *et al.*, 2010), especially in the absence of approved pharmaceutical agents. Despite lifestyle modifications being central to the management of NAFLD, the evidence base upon which these guidelines are based is lacking, and this is particularly true for physical activity and exercise. The focus of this thesis is on defining, exploring and developing this evidence with a view to improving the clinical care of people with NAFLD.

This literature review begins by describing normal metabolic control and the pathophysiology of metabolic disorders. It then focusses on NAFLD and its pathogenesis. The role of physical activity and exercise in maintaining metabolic health is outlined, and the links between these and the development of NAFLD are discussed. The review also includes information on cardiac function in metabolic disease and NAFLD.

1.2 Metabolic Control

Metabolism can be defined as the chemical processes occurring within a cell or organism that are necessary for the maintenance of life. In metabolism some substances are broken down to provide energy for vital processes while others, necessary for life, are synthesized (Frayn, 2003). Tight regulation of these processes by different organs and systems, allows the body to achieve metabolic control. In contrast, even minor deviations in metabolic control can result in the development of metabolic disease, with a significant influence on lifelong health and wellbeing. This section of the literature review will introduce the major metabolic tissues. It will then describe the role of these tissues in metabolic regulation, with a focus on postprandial storage of lipid and carbohydrate, two of the major mediators of metabolic disease.

Given the dependence of the body on lipids for function, it is interesting that at any one time, the total amount of triglyceride in the circulation is around 3g. However, a Western diet contains approximately 100g of fat per day and a typical meal contains 30g of fat. After eating this, the amount of fat found in the circulation could increase dramatically but this is prevented by mechanisms within the body that “buffer” the influx of triglyceride into the circulation and prevent the exposure of tissues to excessive fatty changes (Frayn, 2002). The fine regulation of circulatory lipids is important in order to maintain homeostasis and to direct the lipids to where they are needed. In the human body the major depots for lipid are the liver, skeletal muscle and adipose tissue and are, as such, central to the regulation of circulatory lipids.

Although much larger than the level of circulatory lipids, the amount of free glucose in the body is relatively small - around 12g in the circulation and extravascular space. After eating a typical meal containing 100g of carbohydrate, the influx of glucose could potentially increase the plasma glucose concentration eightfold. This is prevented because coordinated mechanisms come into play to enhance the disposal of glucose from the

plasma and to suppress entry into the circulation of endogenous glucose (Frayn, 2002). After eating a meal high in carbohydrates, the beta-cells of the pancreas increase insulin secretion. Insulin stimulates removal of glucose from blood into skeletal muscle, liver and adipose tissue, and suppresses the entry of glucose from the liver into the blood. The result is that excursions in blood glucose are dampened (Wasserman, 2009). As major depots for the storage and metabolism of carbohydrates, the liver, skeletal muscle and adipose tissue are central to the regulation of circulatory glucose levels.

It is clear that the liver, skeletal muscle and adipose tissue are integral to the maintenance of metabolic homeostasis in the human body. In light of this the independent and coordinated responses of the liver, muscle and adipose tissue, to the postprandial storage of carbohydrate and lipid, their function will now be reviewed.

1.2.1 Metabolic Tissues

The role of the liver in postprandial metabolism

The liver is the first organ to be exposed to the nutrients which enter the body from the intestine after a meal and has a major role in energy storage in the postprandial state. The portal vein is the liver's major blood supply and carries blood which has passed through and around the intestinal tract.

Carbohydrate metabolism in the liver

After a meal, glucose is absorbed from the intestine into the portal vein, exposing hepatocytes to a high concentration of glucose during the absorptive phase. Hepatocytes have mainly GLUT2 glucose transporters which do not respond to insulin, and have a relatively high affinity for glucose so that they normally operate well below saturation. Because there are so many transporters, there is high transport of glucose into the liver. Therefore the rate and direction of movement of glucose across hepatocytes' membrane is determined by the relative glucose concentration in/outside the cell.

When glucose concentration outside the hepatocyte increases, glucose will rapidly be taken into the liver cells and phosphorylated to form glucose 6-phosphate by the enzyme glucokinase. This enzyme has a high affinity for glucose and is not inhibited by its product (glucose 6-phosphate) at a physiological concentration. Like the GLUT2 transporter it has a high capacity and is unaffected, in the short term, by insulin. The presence of the high affinity glucose transporter and the high affinity glucokinase would not alone enable the hepatocytes to take up unlimited quantities of glucose, as glucose 6-phosphate would accumulate in the cell until glucose phosphorylation ceased. Thus there are specific mechanisms for stimulating the disposal of glucose 6-phosphate via glycogen synthesis or glycolysis.

Insulin and glucose both activate the storage of glucose as glycogen. They activate the main regulatory enzyme of glycogen synthesis (glycogen synthase) and inhibit glycogen breakdown (by glycogen phosphorylase). Because insulin reaches the liver directly, and because glucose from the small intestine arrives in the portal vein, the liver can bring about precise control of the system.

Another important function of the liver in glucose metabolism is the synthesis of glucose from other precursors (gluconeogenesis). The substrates for gluconeogenesis are small molecules such as lactate, alanine and glycerol. Gluconeogenesis is stimulated by glucagon and inhibited by insulin. Hepatic gluconeogenesis can be also be stimulated by an increase in the supply of substrate from other tissues. For example, after exercise there are elevated levels of lactate in the blood, some of which will be reconverted to glucose in the liver. During starvation, an increased level of blood glycerol arising from adipose tissue lipolysis will have the same effect. After a meal, hormonal factors will tend to suppress gluconeogenesis whilst substrate supply increases it. This phenomenon is known as the glucose paradox.

Lipid metabolism in the liver

The liver can both oxidise and synthesise fatty acids. Hepatocytes are normally rich in mitochondria and each hepatocyte contains about 800 mitochondria occupying about 18% of the entire liver cell volume (Wei *et al.*, 2008). Mitochondria play an important role in the hepatocytes' metabolism, being the primary site for the oxidation of fatty acids and oxidative phosphorylation.

The liver can oxidise fatty acids by the mitochondrial β-oxidation pathway to produce energy for its many metabolic activities. Fatty acid synthesis and diversion of fatty acids away from oxidation is favoured by high insulin levels. In "fed" conditions, when insulin is elevated, malonyl-CoA levels will be high and fatty acid oxidation will be inhibited (malonyl-CoA inhibits fatty acid entry into the mitochondrion for oxidation). Fatty acids will be diverted into esterification with glycerol 3-phosphate, a process which is stimulated by insulin. Thus in the "fed" state, the liver tends to store fatty acids as triglyceride rather than oxidise them. The hepatic triglyceride pool is not a major energy store for the rest of the body but appears to be a local store for hepatic needs. The stored triglyceride acts as the substrate for hepatic secretion of fat into the bloodstream in the form of vLDL.

In the presence of hyperinsulinemia, the liver converts excess carbohydrate to fat to control blood glucose and prevent hyperglycaemia. Increasing triglyceride concentrations would, in turn, exacerbate IR and set up a vicious cycle. On the whole, enhanced *de novo* lipogenesis (DNL) appears to be a major abnormality of hepatic fat metabolism in subjects with NAFLD (Lavoie and Gauthier, 2006). In healthy human subjects, the contribution of DNL in the liver to IHL in the fasted state is less than 5%. In patients with IR or NAFLD, DNL in the liver is elevated by up to 26% (Tamura *et al.*, 2005). In healthy subjects, DNL is elevated following meals, which can be accounted for by elevation in the circulating levels of lipogenesis precursors. However, in people with NAFLD, DNL is constantly elevated.

Insulin activates the membrane-bound transcription factor sterol-regulatory-element binding protein-1c (SREBP-1c), which transcriptionally activates most genes required for lipogenesis. Lipogenesis is also regulated by glucose: glucose activates the carbohydrate response element binding-protein (ChREBP), which induces expression of liver-type pyruvate kinase, a key regulatory enzyme in glycolysis; this enzyme in turn provides the precursors for lipogenesis. ChREBP also stimulates gene expression of most enzymes involved in lipogenesis (Tamura *et al.*, 2005; Lavoie and Gauthier, 2006; Harrison and Day, 2007). Hyperinsulinemia and hyperglycaemia may also induce these transcriptional factors in humans.

Leptin and adiponectin, the two major fat-derived hormones, have been shown to improve insulin sensitivity and concomitantly reduce IHL (probably by promoting fatty acid oxidation). In the absence of leptin action, lipogenesis is increased and fatty acid oxidation is reduced accounting for the steatosis that occurs in such circumstances. One pathway by which leptin achieves its anti-lipogenic effect in the liver is by lowering expression of SREBP-1c, thus up-regulating genes promoting fatty acid oxidation and down-regulating those involved in lipogenesis (Lavoie and Gauthier, 2006).

The role of skeletal muscle in postprandial metabolism

Skeletal muscle is made up of numerous fibres, and within each fibre there are many myofibrils, themselves highly organised bundles of the proteins actin and myosin. Muscle contraction is brought about through head-groups of myosin filaments binding to the actin filaments. The head-groups can “rock” to move the myosin relative to the actin, detach, and rebind further along the actin. This process requires energy (ATP) which is hydrolysed to release ADP+P and is regulated by calcium binding to a protein known as troponin-C that is associated with the actin filaments.

There are two major types of muscle fibre, referred to by either their contractile properties or colour:

Type 1/Red/Slow twitch fibres: These oxidative fibres have a high concentration of myoglobin, a pigment related to haemoglobin, which assists the diffusion of oxygen into the muscle. They have a high density of capillaries perfusing them and many mitochondria within each cell. These muscle fibres use substrates, largely from the blood, and oxidise them to yield energy. The oxidation of substrates from the blood requires time for diffusion of the substrate to the cell, diffusion of oxygen to the cell, and diffusion out of the cell of carbon dioxide. Therefore contraction of this type of fibre is relatively slow. These fibres are important for sustained, relatively low intensity exercise.

Type 2/White/Fast twitch fibres: These fibres lack myoglobin and are therefore white. They have fewer mitochondria and are more equipped for anaerobic glycolysis than oxidative metabolism. The main substrate for glycolysis is glucose 6-phosphate produced by the breakdown of glycogen stored within the same cells. The sequence of glycogen breakdown and generation of energy by glycolysis can be extremely rapid since everything is “on site”. Their role is to produce energy quickly, but because they are largely dependent upon stored substrate, they cannot maintain this for long. These fibres are important in the rapid generation of energy over short periods.

Skeletal muscle uses both stored fuel (glycogen and triglyceride) and substrates (glucose and fatty acids) taken up from the blood. Glucose uptake is mainly mediated by the insulin-sensitive glucose transporter GLUT4. (GLUT1 is also in skeletal muscle and may play a role in the uptake of glucose at a basal rate). Glucose uptake by GLUT4 has certain characteristics:

- The maximal rate of uptake for GLUT4 is within the physiological range of plasma glucose concentrations.
- In the presence of low concentration of insulin, the maximum activity of glucose uptake is low.
- Increasing insulin concentration brings more transporters into action at the cell membrane and increases glucose uptake.

The glucose may be used for glycogen synthesis or metabolism via glycolysis. Insulin stimulates the enzyme glycogen synthase in muscle and inhibits the enzyme glycogen phosphorylase (Savage *et al.*, 2007). Thus when plasma

insulin is high after a meal, glucose will be stored as glycogen in skeletal muscle.

Free fatty acids, or non-esterified fatty acids (NEFA), are also taken up by skeletal muscle, particularly in the oxidative, slow twitch fibres. Under resting conditions the rate of fatty acid uptake is closely related to the concentration of NEFA in the plasma. Within the cell, fatty acids are oxidised in accordance with their rate of uptake. During exercise, increased blood flow through the muscle increases the delivery of NEFA for oxidation. During the period after a meal, when glucose and insulin levels are high, fatty acid oxidation is restricted and NEFA are diverted into triglyceride synthesis for storage.

The role of adipose tissue in postprandial metabolism

There are two types of adipose tissue, defined essentially in the way that they store triglycerides. Brown adipose tissue gets its colour from large numbers of mitochondria in the cytoplasm and stores lipid in multiple droplets. White adipose tissue stores lipid as one droplet which typically fills most of the cell; the cytoplasm, mitochondria and nucleus are confined to a thin “crust” around the outside. Both types of adipocyte store energy in the form of triglyceride and may release fatty acids when needed by other tissues. The difference is brown adipocytes have a much higher oxidative capacity, and may oxidise a large proportion of the fatty acids released from storage. Brown adipose tissue’s main role is to generate heat. It is highly vascularised and the blood carries heat produced during oxidation to the rest of the body. Humans do not have a significant amount of brown adipose tissue. In the adult human, virtually all adipose tissue is white. Its major role is in the control of the storage and release of fat on a minute by minute basis.

Lipid fuels are not water-soluble and their presence in the plasma is dependent on specialised transport mechanisms. Excess concentrations of fat in the plasma can have adverse consequences (such as atherosclerosis). Therefore

the regulatory role of white adipose tissue is essential to normal health as well as to the coordination of fat metabolism in everyday life, responding to meals and overnight fasting. Fat is stored via two major pathways: 1. uptake of the triglyceride from the plasma; 2. *de novo* lipogenesis (the synthesis of lipid from other sources). On a typical Western diet, high in fatty acids, route one is the most important (Savage *et al.*, 2007).

Fat in the plasma is present in lipoprotein particles. The largest of these particles (chylomicrons) are too big to escape from the capillaries into the intestinal fluid; therefore the adipocytes cannot take them up directly. Adipocytes produce the enzyme lipoprotein lipase (LPL) which hydrolyses the triglyceride in lipoprotein particles to release NEFA, which can then diffuse into the interstitial space and so reach the adipocytes (Frayn *et al.*, 1994). Since LPL must act in the capillaries, it is exported from the adipocytes to the endothelial cells lining the capillaries of the adipose tissue. LPL can thus come into contact with, and act upon, passing chylomicrons thus hydrolysing their triglyceride and releasing NEFA (Sniderman *et al.*, 1998). Activity of LPL in adipose tissue is stimulated by insulin, secreted in response to an increase in blood glucose levels. After a typical meal containing fats and carbohydrates, the uptake of fat into adipose tissue will be stimulated. The role of LPL will be discussed again in Section 1.5.1 with respect to physical inactivity.

The rate at which LPL can hydrolyse lipoprotein triglycerides is determined by the number of active LPL molecules in contact with that particle. It is also influenced by the speed at which NEFA that are produced can be removed from the capillary microenvironment. If fatty acids are not removed, capillary concentrations will rise abnormally, LPL activity will be inhibited (by its product), and lipolysis will be reduced (Sniderman *et al.*, 1998). Under normal circumstances, approximately half of the fatty acids released from the chylomicrons are trapped immediately in the adipocytes, and half enter the general circulation (Sniderman *et al.*, 1998). Once inside the cell, the fatty acids are esterified to form triglyceride which joins the lipid droplet for storage.

The mobilisation of fat involves the hydrolysis of stored lipid and is also called lipolysis (the breakdown of triglyceride to fatty acids). This is catalysed by hormone-sensitive lipase (HSL). Insulin helps to control the action of HSL by promoting fat storage and restraining fat mobilisation as necessary (Frayn *et al.*, 1994). In the fasting state, when the intracellular enzyme HSL is active, there is a large net outflow of fatty acids from adipocytes into the plasma, and the concentration gradient will not favour fatty acid uptake. In the postprandial state, HSL is suppressed by insulin and the pathway of fatty acid esterification is stimulated (Frayn *et al.*, 1994; Frayn, 2002).

Adipocytes take up excess fatty acids in the short term. Normally the uptake of fatty acids after a meal will be balanced by fat mobilisation in the post-absorptive state (e.g. during the night-time fast) and during exercise, so that in most people the size of fat stores remains relatively constant. If there is a long-term positive energy balance, adipocytes increase in size and number to increase fat storage, and overall fat mass increases, resulting in weight gain.

1.3 Pathophysiology of Metabolic Disorders

Although circulating levels of carbohydrates and lipids are generally well maintained, a loss of control results in the development of metabolic disease. This section will now review the relative roles of carbohydrate and lipid in the development of metabolic disease and with this, describe the contributions of the liver, skeletal muscle and adipose tissues.

1.3.1 Type 2 Diabetes, Insulin Resistance and Obesity

Insulin is released from the beta-cells of the pancreas in response to increases in blood sugar. This stimulates glucose uptake and storage in muscle and adipose tissue while at the same time suppressing the production of glucose in the liver (Gulve, 2008). When blood sugar falls below normal levels, the alpha-cells of the pancreas secrete glucagon (the opposing hormone of insulin) to normalise blood sugar concentration. Glucagon increases blood glucose concentration by stimulating the liver's glycogenolytic and gluconeogenic pathways.

Insulin resistance (IR) is the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells (Goodpaster and Wolf, 2004). IR in muscle cells decreases glucose uptake and so decreases local storage of glucose as glycogen. It is also accompanied by an increase in intramuscular lipid. IR is arguably the earliest detectable and dominant metabolic defect in patients developing Type 2 diabetes (T2DM) and NAFLD.

T2DM is a condition characterised by high blood glucose levels caused by relative insulin deficiency or the body's inability to use insulin efficiently (i.e. their body has become insulin resistant). It generally occurs in those who are obese, sedentary and over 45 years of age. Humans with T2DM exhibit the classic triad of hyperinsulinemia, hyperglycaemia and hypertriglyceridemia (Toledo *et al.*,

2006; Brown and Goldstein, 2008). High blood glucose levels in the face of high levels of insulin are attributed to IR. The prevalence of diabetes worldwide was estimated to be 2.8% in 2000 and predicted to be 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al.*, 2004).

People with IR or T2DM are limited in their capacity to dispose of glucose to storage depots in the postprandial phase. Although overall tissue glucose uptake is essentially “normal” in a quantitative sense, postprandial plasma glucose levels are higher in people with IR or T2DM (Woerle *et al.*, 2006). The amount of glycogen accumulation in the liver and skeletal muscles of these patients was found to be lower, despite the “normal” overall tissue glucose uptake, suggesting that glucose might be taken up excessively by tissues that are not normally major sites of postprandial glucose disposal (Woerle *et al.*, 2006).

IR and T2DM have been closely linked to obesity. Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. Body mass index (BMI), a measurement which compares weight and height, defines people as overweight (pre-obese) if their BMI is between 25 and 30 kg/m^2, and obese when it is greater than 30 kg/m^2. Worldwide, 1.1 billion adults and 10% of children are classified as overweight or obese (Haslam and James, 2005).

In obesity, IR is induced by fat deposited intracellularly in muscle, liver and adipose tissue, and by the excretory products of the expanded adipocyte mass. These products include inflammatory markers, including tumour necrosis factor-α (TNF-α), which suppresses the secretion of adiponectin. Adiponectin is a powerful insulin sensitiser, which is secreted less as the adipocyte mass expands. As adipose tissue becomes “full” and unable to store increasing circulatory fatty acids, fat infiltrates other organs. In the pancreas, this excess

fat can damage the beta cells, and reduces the islets' capacity to maintain the increased insulin output demanded by IR, so glucose intolerance and premature T2DM readily develop (Haslam and James, 2005). Ectopic fat is also deposited in the liver resulting in a predisposition to NAFLD (Tamura *et al.*, 2005).

Metabolic abnormalities which are usually associated with obesity, do not, however, affect all obese people. Approximately 10-25% of obese people and a fraction of morbidly obese individuals are not affected by metabolic disturbances. These metabolically "healthy-obese" subjects are insulin sensitive, have normal blood pressure, a favourable lipid profile, a lower proportion of visceral fat, less liver fat and a normal glucose metabolism despite having an excessive amount of body fat (Pajunen *et al.*, 2011). However, despite awareness of the healthy-obese phenotype, there currently exist no established criteria by which to define these individuals. The defining characteristics of the metabolically healthy-obese phenotype, in contrast to obese individuals with metabolic risk, include limited abdominal, particularly visceral fat accumulation, an earlier onset of obesity (<20 years) and high levels of physical activity.

1.3.2 The role of skeletal muscle

Skeletal muscle represents the primary site of insulin-mediated glucose disposal and most glucose that is cleared from the blood in response to insulin is stored as glycogen in skeletal muscle (Gulve, 2008). When insulin-stimulated glucose transport into skeletal muscle is decreased (as it is in people with IR and T2DM) the result is an inability to keep blood glucose concentrations within normal limits. Thus skeletal muscle plays a primary role in the maintenance of normal blood glucose concentrations (Turcotte and Fisher, 2008).

Skeletal muscle has large numbers of mitochondria and is heavily reliant on oxidative phosphorylation for generating ATP from carbohydrates (mainly

glucose and glycogen) and fat based fuels. Mitochondrial oxidative phosphorylation provides up to 90% of cellular ATP. A mismatch between mitochondrial oxidative capacity and the capacity for glycolysis may be an important factor in the development of IR. Mitochondrial content, mitochondrial function and oxidative capacity are decreased in people with IR and T2DM (Petersen *et al.*, 2004; Hawley and Lessard, 2007) and can lead to a decrease in fatty acid oxidation within the cells. Impairments in muscle oxidative capacity could arise from a defect in mitochondrial function and/or a decrease in the number of mitochondria.

Fatty acid uptake capacity may be inherently high in IR muscle. Fatty acid flux across the plasma membrane occurs through a highly regulated, protein-mediated process that involves one or several fatty acid transporter proteins (e.g. FABPpm, CD36). High rates of fatty acid uptake in people with IR are associated with an increased total protein content of FABPpm and with a permanent relocation of CD36 to the plasma membrane. Thus a higher plasma membrane content of FABPpm, CD36, or both may provide a cellular mechanism through which rates of fatty acid uptake are increased in IR muscle (Turcotte and Fisher, 2008). Low CD36 content in mitochondrial membranes, defects in the ability of muscle to translocate CD36 to mitochondrial membranes or both are important factors regulating low fatty acid oxidative capacity associated with IR. Therefore, in people with IR, there is increased uptake of fatty acids within the muscle, but an inability to transfer these fatty acids into the mitochondria for oxidation and thus there is an accumulation of fatty acids within the muscle (Turcotte and Fisher, 2008). Measurements of intramuscular lipid (IML) correlate more closely with IR than any other commonly measured indices (Savage *et al.*, 2007). IR in muscle was accompanied by an increase of approximately 80% in IML content in the IR subjects, compared with insulin-sensitive controls (Petersen *et al.*, 2004). Increases in IML could occur as a result of the increased delivery of fatty acids from lipolysis and/or decreased rates of mitochondrial oxidative phosphorylation.

Mitochondrial rates of ATP production were reduced by approximately 30% in the muscle of the IR subjects, compared with insulin-sensitive controls (Petersen *et al.*, 2004). IR skeletal muscle is characterised by lower oxidative capacity and lower post-absorptive rates of fatty acid oxidation (Goodpaster *et al.*, 2001). This raises the possibility that the association between lipid accumulation within muscle and IR is influenced by a lower capacity for the oxidation of lipid as an energy substrate. Studies have identified increases in plasma fatty acid concentrations and IML content in the IR offspring of patients with T2DM, suggesting that dysregulation of fatty acid metabolism may mediate the IR in these people. IR in the offspring of patients with T2DM is due to dysregulation of intramyocellular fatty acid metabolism, which may be caused by an inherited defect in mitochondrial oxidative phosphorylation. Such a defect might be due to a reduction in mitochondrial content, which in turn might be attributable to a reduced ratio of type 1 to type 2 muscle fibres (Petersen *et al.*, 2004). Since mitochondria have a critical role in mediating glucose-induced insulin secretion, the presence of similar inherited defects in beta-cell mitochondrial function or content, in the setting of peripheral IR, might explain the increased incidence of diabetes in the IR offspring of patients with T2DM.

1.3.3 The role of adipose tissue

Adipose tissue is the largest endocrine organ in the body and does more than just store excess energy. Adipocytes release numerous hormones and other signalling factors that travel throughout the body sending messages to the musculoskeletal system, pancreas, liver, heart, adrenal glands and central nervous system. In people with excess body fat, adipocytes release abnormal factors that can cause or amplify metabolic disorders. Thus, adipose tissue itself plays a role in causing diseases such as T2DM and cardiovascular disease (CVD) (Stehno-Bittel, 2008).

Impairments in adipose tissue function will lead to less effective buffering of fatty acid fluxes in the circulation and thus an increase in postprandial NEFA and triglyceride concentrations. Loss of adipose tissue's ability to buffer the

incoming flux of fatty acids will inevitably lead to increased exposure of other tissues to this increased flux (Frayn, 2002). A larger adipose tissue mass delivers more NEFA to the systemic circulation as the ability of insulin to suppress fatty acid release is impaired (Frayn, 2002). These “extra” fatty acids compete for substrate utilisation in skeletal muscle, which in turn decreases glucose utilisation. This increases blood glucose concentration and provides the stimulus for increased insulin secretion and hyperinsulinaemia is a key feature of the IR syndrome (Karpe and Tan, 2005).

However, there is significant evidence to suggest that adipose tissue is insulin sensitive and hyperinsulinaemia may therefore lead to a constant lipolytic inhibition in adipose tissue. Consequently, the main function of adipose tissue, to rapidly switch between fat uptake and fat release, will be hampered (Frayn, 2002). Adipose tissue blood flow is the conveyor of signals and substrates to and from the adipose tissue. In healthy people, adipose tissue blood flow is increased markedly by food intake, whereas in IR subjects this response is blunted. This is another facet of the unresponsiveness of adipose tissue in the IR syndrome.

Different fat compartments are associated with different metabolic risks. Abdominal fat carries the greatest health risk, however visceral fat is the most important predictor of metabolic disorders. Visceral adipose tissue (VAT) is a stronger correlate of CVD risk than BMI, waist circumference, or subcutaneous adipose tissue (SAT). Central obesity, in which fat mass is predominantly intra-abdominal, is more strongly associated with IR, dyslipidemia and atherosclerosis than is peripheral obesity, in which fat is predominantly gluteofemoral (Porter *et al.*, 2009).

Multiple small studies have demonstrated that the visceral fat compartment is metabolically active, secreting such vasoactive substances as inflammatory markers, adipocytokines, markers of haemostasis and fibrinolysis, and growth factors, which may contribute to its role in cardiometabolic risk factor

manifestation (Fox *et al.*, 2007). Weight loss through diet and exercise, which results in reductions in visceral fat, is associated with improvements in IR, blood pressure, serum lipids and inflammatory markers.

In contrast to the detrimental effects of VAT, studies have a highlighted a possible protective role for SAT. In humans, increased subcutaneous leg fat is associated with a reduced risk of disturbed glucose metabolism and dyslipidemia, independent of abdominal fat (Porter *et al.*, 2009). Thiazolidinedione treatment, which increases total fat mass, mostly in subcutaneous fat stores, improves insulin sensitivity (Ravikumar *et al.*, 2008). Surgical removal of VAT results in decreased glucose and insulin levels in humans, while removal of SAT by liposuction does not always result in improvements in glucose metabolism or lipid levels. Transplantation of subcutaneous fat into visceral compartments in mice produces decreases in body weight and total fat mass and improved glucose metabolism, suggesting that subcutaneous fat may be intrinsically different from visceral fat in ways that are beneficial (Porter *et al.*, 2009).

Inter-muscular adipose tissue is visible adipose tissue beneath the muscle fascia and between muscle groups. It is associated with a decrease in insulin sensitivity in individuals with T2DM, suggesting that these individuals benefit from minimal inter-muscular adipose tissue. Regional adiposity has an adverse effect on the function of the insulin receptors within the muscle and is negatively associated with insulin sensitivity through cytokine mediated pathways (Hilton *et al.*, 2008).

One possible explanation for obesity-related cardiometabolic disease is the portal vein hypothesis, which proposes that increased visceral fat leads to higher NEFA concentrations in the portal vein, increased systemic fatty acid flux, and increased hepatic lipase activity, which removes lipids from LDL and HDL, and may lead to dyslipidemia (Porter *et al.*, 2009). The ectopic fat hypothesis suggests that a characteristic of obesity is fat deposition in liver,

skeletal muscle, and pancreatic beta-cells resulting from insufficient adipocytes growth and differentiation in the setting of nutritional excess. Such ectopic fat stores are theorized to impact tissue and organ function by physical compression, the secretion of various locally acting substances, and cell dysfunction or cell death of non-adipose cells a phenomenon known as lipotoxicity. In line with this theory, SAT represents a proper expansion of non-pathogenic adipocytes and therefore may be considered a protective fat depot. Improvements in insulin sensitivity with thiazolidinedione treatment, which increases subcutaneous fat stores, are suggestive of a protective effect of SAT (Porter *et al.*, 2009).

Obesity is the consequence of both an enlargement of adipocytes and an increase in the number of adipocytes. Larger fat cells are less sensitive to insulin and exert a higher basal rate of lipolysis than small fat cells (Frayn, 2002; Stehno-Bittel, 2008). As adipocytes enlarge to store excess fat, their efficiency as “buffers” decreases. Excess adipose tissue initiates several cellular pathways which lead to chronic inflammation. The larger number of adipocytes associated with obesity causes a local oxygen shortage triggering a state of chronic hypoxia and local inflammation in the surrounding cells. Adipocytes themselves can stimulate inflammatory responses. Locally secreted adipokines attract proinflammatory macrophages into adipose tissue, where they encircle dying adipocytes. The macrophages release their own factors that further stimulate the inflammatory process. Obesity is often accompanied by increased levels of NEFA which could activate proinflammatory responses in blood vessels, fat cells and immune cells (Stehno-Bittel, 2008).

If excessive adipose tissue leads to inflammation, how does the inflammation in fat cells lead to IR? The inflammatory process is first activated by macrophages within adipose tissue. Those signals spread in a paracrine fashion, activating inflammation in nearby tissue and resulting in IR in those tissues. NEFA are subsequently released from stimulated adipocytes, leading to secondary IR at distant sites such as skeletal muscle (Stehno-Bittel, 2008). Adipocytes are important in maintaining the level of oxidative stress in the body. Oxidative

stress is caused by free oxygen radicals, which are super-reactive oxygen molecules. Normally low levels of free oxygen radicals are a necessary part of cellular respiration and are balanced by dietary antioxidants. In obesity, the balance is lost and excess free oxygen radicals accumulate in the tissues. Oxidative stress hastens complications of obesity, including IR and T2DM (Stehno-Bittel, 2008).

1.3.4 The role of the liver

The liver stores glucose after food ingestion, and releases glucose to the circulation between meals in order to maintain appropriate plasma glucose levels (Gulve, 2008). In T2DM, excessive hepatic glucose output contributes to fasting hyperglycaemia, with increased gluconeogenesis being the predominant mechanism. Excess glycogen accumulation in the liver is seen in 80% of diabetics and hepatic fat accumulation affects 40-70% (Levinthal and Tavill, 1999). Fat is stored as triglyceride and may be a manifestation of increased fat transport to the liver, enhanced hepatic synthesis and decreased oxidation or removal of fat from the liver.

Metabolic dyslipidemia is characterised by high circulating triglycerides and low HDL levels and is frequently accompanied by hepatic steatosis (Toledo *et al.*, 2006). Increased hepatic lipogenesis contributes to both of these problems. Because insulin fails to suppress gluconeogenesis but continues to stimulate lipogenesis in both obese and lipodystrophic IR mice, it has been proposed that a selective post-receptor defect in hepatic insulin action is central to the pathogenesis of fatty liver and hypertriglyceridemia in these mice (Semple *et al.*, 2009). In humans, IR subjects had a marked defect in muscle glycogen synthesis and diverted much more of their ingested energy into hepatic *de novo* lipogenesis, resulting in increased plasma triglycerides, decreased HDL and increased triglyceride synthesis. vLDL production was also seen to increase (Petersen *et al.*, 2007). These factors may predispose these individuals to NAFLD and CVD.

Under normal conditions, dietary glucose stimulates insulin secretion from the pancreas. The insulin travels directly to the liver via the portal vein, where it elicits two key actions at the level of gene transcription. Firstly, insulin stimulates the phosphorylation of Fox01, a transcription factor that activates gluconeogenesis. Insulin-stimulated phosphorylation prevents Fox01 from entering the nucleus, and hence it down regulates genes required for gluconeogenesis, most prominently phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatease (G6Pase). The result is a decrease in hepatic glucose output which helps to keep blood glucose low. Secondly, insulin activates the transcription factor SREBP-1c which enhances transcription of genes required for fatty acid and triglyceride biosynthesis, most prominently acetyl-coenzyme A carboxylase (ACC) and fatty acid synthase (FAS) (Brown and Goldstein, 2008). The newly produced triglycerides are secreted in vLDL, which delivers triglycerides to adipose tissue for storage and to muscle for combustion. Uptake of vLDL-derived fatty acids in adipose tissue is facilitated by insulin, which increases the amount of LPL on the surface of endothelial cells (Brown and Goldstein, 2008).

In people with T2DM, IR or obesity these pathways can become impaired. In the liver, the Fox01 pathway becomes IR. Despite extremely high insulin levels, the mRNAs for PEPCK and G6Pase remain high and gluconeogenesis continues. Despite IR in the Fox01 pathway, insulin sensitivity is maintained in the SREBP-1c pathway. Thus, nuclear SREBP-1c levels are extremely high, fatty acid synthesis is increased, and high levels of triglycerides accumulate in the liver. Excess triglycerides are secreted in vLDL, raising plasma triglyceride levels (Brown and Goldstein, 2008; Semple *et al.*, 2009). Fatty acids derived from these triglycerides worsen the IR state in muscle and adipose tissue. The net result is the classic T2DM triad of hyperglycaemia, hyperinsulinaemia and hypertriglyceridemia. Excess triglycerides accumulating in the liver can lead to NAFLD. Some of the excess triglycerides also deposit in the beta-cells of the pancreas, where they contribute to the eventual beta-cell failure that leads to frank diabetes (Brown and Goldstein, 2008).

1.4 Non-alcoholic fatty liver disease

The liver plays a central role in both glucose and lipid regulation and as a result is influential in the development of metabolic disease. Consequently, metabolic deregulation in the liver has significant clinical implications. This section will define the commonest liver complication, non-alcoholic fatty liver disease, review the literature on prevalence and also the clinical presentations.

1.4.1 Defining non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) occurs in developed and developing countries and is present across a full range of ethnicities, such that it is now considered to be the most common liver condition in the world (Smith and Adams, 2011). NAFLD represents a spectrum that spans from asymptomatic steatosis to potentially life-threatening non-alcoholic steatohepatitis (NASH). NASH is distinguished from simple steatosis by the presence of hepatocyte injury, inflammation and fibrosis (Church *et al.*, 2006) and although simple steatosis is a relatively benign condition, NASH can progress to cirrhosis, liver cancer and liver failure. Once cirrhosis develops, patients are at high risk of developing hepatic decompensation and of dying from a liver-related cause. NAFLD has become an increasingly common indication for liver transplant (Erickson, 2008).

When intrahepatic lipid (IHL) content exceeds 5% of the liver's total weight, it is clinically defined as having excess lipid. The 5% liver fat cut-off used to define NAFLD was established from a study in the general population using proton magnetic resonance spectroscopy to determine the "upper limit of normal" of liver fat (Szczepaniak *et al.*, 2005). If IHL increases above 10%, small globules of fat begin to appear in many of the hepatocytes - when IHL exceeds 30%, virtually all the hepatocytes are affected and contain a large droplet of fat (Ueno *et al.*, 1997). Excess IHL is caused by a failure of normal hepatic fat metabolism. This is due to an imbalance between fat arriving at the liver and fat being exported from or oxidised by the liver.

1.4.2 Epidemiology

The prevalence of NAFLD in Western countries is estimated to be around 20-30% (Harrison and Day, 2007) but most patients remain asymptomatic and undiagnosed, making precise predictions about disease prevalence difficult. In people with T2DM, this figure increases to 70-75% (Targher *et al.*, 2007). In morbidly obese patients undergoing bariatric surgery, approximately 90% have NAFLD and 36-37% have NASH (Harrison and Day, 2007).

1.4.3 Clinical Presentations

The majority of patients with NAFLD are asymptomatic, although some complain of non-specific fatigue and weakness, and others have described vague right-sided abdominal pain. The diagnosis of NAFLD, anywhere on the spectrum, rests upon three key features (James and Day, 1998):

1. Histopathological features, of which the presence of fat and of alcohol-like liver damage are essential.
2. Rigorous exclusion of alcohol as a cause for the disease.
3. Appropriate investigations to exclude other forms of chronic liver disease.

Most patients are diagnosed after liver function tests are performed for another medical reason, and results demonstrate an abnormality in liver enzymes. People with NAFLD usually display persistently raised liver enzymes, although the level of elevation does not indicate the underlying disease severity (Mofrad *et al.*, 2003; Szczepaniak *et al.*, 2005; Fracanzani *et al.*, 2008). NAFLD is by far the most likely histological diagnosis in the increasing number of patients presenting to liver clinics with persistently abnormal liver function tests (Day, 2002) and it has been reported that over two-thirds of patients presenting with unexplained abnormal liver function tests will have NAFLD (Day, 2006). Risk factors for developing NAFLD include increasing age, being overweight/obese, a sedentary lifestyle, T2DM or IR, hyperlipidemia, diet and family history.

Ultrasonography, computer tomography and magnetic resonance imaging are accepted modalities for detecting moderate to severe (>30%) hepatic steatosis. The major limitation of these imaging modalities are that they cannot

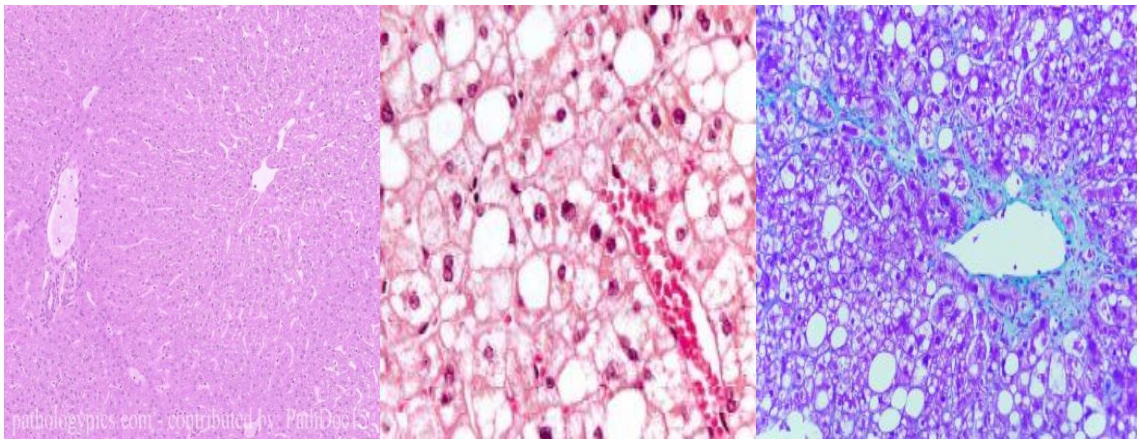
differentiate between the histological subtypes of simple steatosis or NASH, nor can they stage the degree of fibrosis (Saadeh *et al.*, 2002). Modern techniques, such as proton magnetic resonance spectroscopy (^{1}P-MRS), quantify IHL accurately and non-invasively but its use is currently limited to research settings due to cost (Smith and Adams, 2011).

The gold standard for diagnosing NAFLD is a liver biopsy, however, it is impractical to subject all patients with mild elevations of liver enzymes to liver biopsy due to its associated morbidity and mortality and also the lack of current treatment strategies. The main purpose for performing a liver biopsy is to determine the stage of the disease (Day, 2006) – see Figure 1. Biopsies can be staged using a number of scales. A five-stage scale was developed by Brunt et al (1999) which incorporates all degrees of liver damage: stage 0 - absence of fibrosis; stage 1 - perisinusoidal or portal fibrosis; stage 2 - perisinusoidal and portal/periportal fibrosis; stage 3 - septal or bridging fibrosis; stage 4 – cirrhosis (Brunt *et al.*, 1999). The NAFLD Activity Score (NAS) specifically includes only features of active injury that are potentially reversible in the short term. The score is defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2); thus ranging from 0 to 8. Fibrosis, which is both less reversible and generally thought to be a result of disease activity, is not included as a component of the activity score (Kleiner *et al.*, 2005). The separation of fibrosis from other features of activity is an accepted paradigm for staging and grading for both NASH and chronic hepatitis. This score may be useful in intervention studies, as it is more likely to demonstrate subtle changes in histology as a result of therapy.

Most hepatology clinics restrict liver biopsy to patients with some of the following: alanine aminotransferase (ALT) greater than twice the normal; aspartate aminotransferase (AST) greater than ALT; moderate “central” obesity; T2DM or impaired glucose tolerance; and hyperlipidemia (Day, 2002). Histopathologically, NAFLD presents in exactly the same way as alcoholic liver disease and it is the strict assessment of alcoholic intake which determines which category a patient is placed in. To help distinguish between NAFLD and alcoholic liver disease, the ratio of ALT to AST is almost always greater than 1

in NAFLD (where ALT exceeds AST) and almost always less than 1 in alcoholic liver disease. However, as hepatic fibrosis progresses in NAFLD this ratio can be altered as ALT levels may fall so interpretation of this ratio must be taken with care.

Figure 1: Liver biopsies showing (from left-right) normal liver, fatty liver and liver fibrosis



Long-term prognosis depends on the histological stage of disease at presentation. Patients with simple steatosis have a relatively benign “liver” prognosis; their risk of developing clinical evidence of cirrhosis over 15-20 years is 1-2%. Patients with NASH and fibrosis can progress to cirrhosis with a risk varying from 0% at 5 years to 12% over 8 years (Day, 2006). Once cirrhosis develops, patients are at a high risk of developing hepatic decompensation and of dying from a liver-related cause, including liver cancer.

1.4.4 The Pathogenesis of NAFLD

It is now recognised that hepatic steatosis (the initial stage of NAFLD) should be targeted early to prevent complications associated with the progression of this condition. However, disease progression appears to vary considerably across the NAFLD population and some people with fatty liver may never go on to develop NASH, while some show a rapid deterioration in liver health. A two-hit hypothesis has been proposed (Day and James, 1998) to explain this inconsistent progression of NAFLD to NASH in humans. Hepatic steatosis

represents the initial insult, or “first hit” that causes the first stage of NAFLD. These “fatty” hepatocytes then become vulnerable to added insults, or “second hits” that increase hepatic oxidative stress, which may lead to the progression from steatosis to NASH. Possible second hits may include bacterial infections, toxins or hormonal changes.

The primary event of NAFLD is the accumulation of fat in the hepatocytes. This fat comes from several possible sources (see Figure 2):

- increased fatty acid delivery to the liver as a result of high dietary fat intake

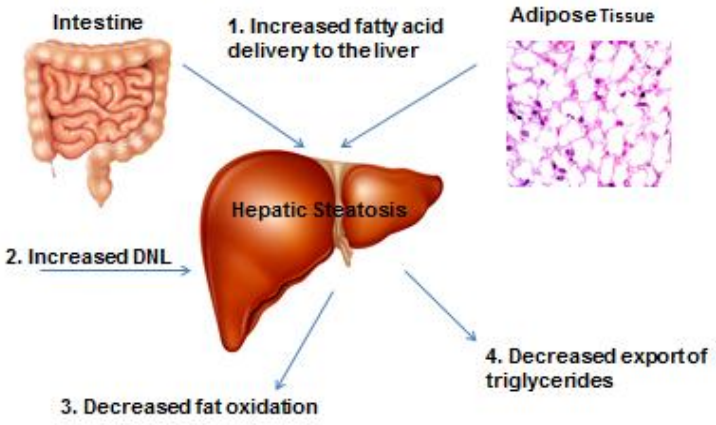
- increased lipolysis within IR adipose tissue releasing more fatty acids into the portal vein for uptake by the liver

- increased hepatic *de novo* lipogenesis (DNL)

- decreased free fatty acid oxidation

- decreased export of triglyceride from the liver

Figure 2: The primary event in NAFLD is the accumulation of fat in hepatocytes



An increased circulating plasma NEFA pool seems to be a major determinant in the pathogenesis of NAFLD and accounts for approximately 60% of the IHL in

NAFLD patients (Lavoie and Gauthier, 2006). In IR states, insulin does not fully suppress the activity of HSL in adipose tissue, which results in enhanced lipolysis and release of fatty acids into the circulation. Uptake of fatty acids by the liver is not regulated and, as a result, plasma NEFA concentration is directly related to the influx of fatty acids to the liver. Thus, the overproduction of fatty acids by adipose tissue that flows to the liver via the NEFA pool is the most likely explanation for excess triglyceride accumulation in NAFLD (Tamura *et al.*, 2005). This would be in line with the concept that the liver acts as a buffer for the influx of fatty acids. Mobilised fatty acids produced from the lipolysis of VAT are directly trafficked through the liver via portal circulation making it no surprise that increased visceral adiposity is strongly correlated to fatty liver (Church *et al.*, 2006).

High levels of circulatory insulin and glucose up-regulate SREBP-1c and ChREBP expression in the liver respectively (Tamura *et al.*, 2005; Lavoie and Gauthier, 2006). These proteins transcriptionally activate most genes required for lipogenesis. In healthy human subjects, hepatic DNL contributes approximately 5% of IHL in the fasted state and 18-23% after a meal (Timlin and Parks, 2005). In those with NAFLD, DNL is constantly elevated, contributing approximately 26% of IHL irrespective of feeding state (Donnelly and Smith, 2005). Elevated circulating triglycerides exacerbate this problem by impeding insulin stimulated glucose uptake (Ferrannini *et al.*, 1983). Thus creating a vicious cycle where elevated IHL levels impede hepatic insulin action, causing increased portal insulin levels and further increasing IHL (Taylor, 2008).

Two major fat-derived hormones, leptin and adiponectin, have been shown to improve insulin sensitivity and reduce IHL, probably by promoting fatty acid oxidation (Lavoie and Gauthier, 2006). Leptin is an adipokine originating mainly from white adipose tissue that plays an important role in regulating food intake, energy expenditure and adiposity. Despite its role in centrally regulating appetite, leptin also acts peripherally on several target tissues, including liver, and protects them against fat accumulation by enhancing fat oxidation (Yasari *et al.*, 2009). In the absence of leptin action, lipogenesis is increased and fatty

acid oxidation is reduced resulting in steatosis. One pathway by which leptin achieves its anti-lipogenic effect in the liver is by lowering expression of SREBP-1c, thus up-regulating genes promoting fatty acid oxidation and down-regulating those involved in lipogenesis (Lavoie and Gauthier, 2006).

In summary, lipid accumulates in the liver as a result of increased uptake from dietary fat and increased lipolysis releasing fatty acids from an enlarged adipose tissue store; DNL increases liver fat by manufacturing lipid from non-lipid precursors. Net lipid output is decreased due to decreased NEFA oxidation and decreased export of triglycerides into the circulation. In combination, the changes in circulatory substrates have a profound impact upon liver metabolism, promoting the accumulation of lipid in the liver and sustained glucose output. These changes in metabolic control create a vicious circle that elevates circulatory glucose and insulin, results in a sustained elevation in circulatory lipids, and in turn, promotes further accumulation of lipid in the liver. If sustained these changes will have a significant impact upon the development of metabolic complications such as T2DM, cardiovascular disease and even the advancement of liver disease itself.

Differences in prevalence, clinical profile, histological severity and outcome of NAFLD in different ethnic groups suggests a genetic contribution accompanying the clear environmental role. This has prompted investigation of polymorphisms of several genes, including those involved in lipid handling (lipolysis, triglyceride synthesis), insulin signaling, oxidative stress and hepatic fibrosis. A genetic variant in patatin-like phospholipase domain-containing protein 3 (PNPLA3) has been identified as a strong predictor of hepatic fat content (Romeo *et al.*, 2008). PNPLA3 is associated with the endoplasmic reticulum and with lipid droplets in hepatoctyes, however, its function remains unknown. In a large (n=9,229), multiethnic population study, the PNPLA3 allele was most common in Hispanic people, the group most susceptible to NAFLD (Romeo *et al.*, 2008). Furthermore, IHL content was over two times higher in PNPLA3 homozygotes than in non-carriers (Romeo *et al.*, 2008). As such, it is clear that environmental and lifestyle influences play a significant role in the development and

progression of NAFLD, however, genetic factors may also be important in determining the susceptibility to NAFLD and its progression to cirrhosis. Importantly, the gene-environment interactions in this area are yet to be determined.

1.5 Physical Activity, Exercise and Metabolic Health

Although much attention has historically been given to the role of nutrition in the management of obesity and NAFLD, emerging evidence suggests that energy expenditure also plays an integral role in adequate metabolic control. Our everyday lives consist of activities which, without us paying conscious effort, have a profound impact upon our health and wellbeing. Typically the activities of the day can be broken into four distinct categories; 1) inactivity or sedentary behaviour, 2) physical activity, 3) exercise, and 4) sleep. These categories will now be discussed before the impact of them upon metabolic health is reviewed.

The definition of being physically inactive or sedentary is controversial. Some groups define inactivity as expending less than 1.5 kcal/kg/day in leisure physical activities (National Population Health Survey of Canada: www.hc-sc.gc.ca/fn-an/surveill/nutrition/population/index-eng.php), while the UK National Obesity Forum indicates that 3000-6000 steps/day is sedentary or inactive (www.national obesityforum.org.uk). In the US National Health Interview Survey, adults were classified as inactive if they did not report any sessions of light to moderate or vigorous leisure-time physical activity of at least 10 minutes a day (www.cdc.gov/nchs/nhis). Sedentary behaviour is not simply a lack of physical activity but is a cluster of individual behaviours where sitting or lying is the dominant mode of posture and energy expenditure is very low. Sedentary behaviours are multi-faceted and might include behaviours at work or school, at home, during transport and in leisure-time. Typically, key sedentary behaviours include screen-time (TV viewing, computer use), motorised transport and sitting.

Physical activity is defined as “any bodily movement produced by contraction of skeletal muscles and resulting in energy expenditure above the basal level” (Wittink *et al.*, 2011) and constitutes many of the activities carried out as part of the daily routine. The term "physical activity" should not be confused with "exercise". Exercise is a subcategory of physical activity in which planned,

structured and repetitive bodily movements are performed to maintain or improve physical fitness. Physical activity includes exercise as well as other activities which involve bodily movement and are done as part of playing, working, active transportation, house chores and recreational activities.

A physical activity can be defined in terms of its metabolic equivalent (MET) level, a physiological measure expressing the energy cost of the task. It is defined as the ratio of metabolic rate (and therefore the rate of energy consumption) during a specific physical activity to a reference metabolic rate, set by convention to 3.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ or equivalently 1 $kcal \cdot kg^{-1}\ h^{-1}$ or 4.184 $kJ \cdot kg^{-1}\ h^{-1}$ (Ainsworth *et al.*, 2000). 1 MET is considered as the resting metabolic rate (RMR) measured during quiet sitting. Activities of less than 3 METs are classed as “light” (e.g. desk work, watching television, slow walking), 3-6 METs as “moderate” (e.g. walking at 3-4mph, cycling less than 10mph), and over 6 METs as “vigorous” (e.g. running, circuit training).

With sleep playing an important role in physiological and cognitive wellbeing, alongside the large proportion of our lives which is spent asleep, it is not surprising that variations in sleep, whether duration or pattern, influence metabolic and mental health. Cross sectional and prospective cohorts reveal that self-reported sleep duration of less than seven hours is associated with an excess risk of cardiovascular (CV) disease (up to 33%), Type 2 diabetes (T2DM) and all-cause mortality (Ayas *et al.*, 2003; Tamakoshi *et al.*, 2004). However, as the objective of the present work is on physical inactivity, physical activity and exercise, this review will focus on these.

This literature review will now explore the links between physical inactivity, physical activity, exercise and metabolic control, and will consider different methods of assessing physical activity levels.

1.5.1 Physical Inactivity and Metabolic Control

Subtle changes in sedentary behaviour may contribute to obesity and metabolic disorders, potentially as much as lack of moderate-vigorous physical activity. Physical inactivity has been identified as the fourth leading risk factor for global mortality (6% of deaths globally) (WHO, 2003) and physical inactivity alone was estimated to cost the NHS £1.06billion in 2002 (Allender *et al.*, 2007). Even if adults meet the public health guideline for leisure-time physical activity, they may have a high risk of becoming overweight or developing metabolic disorders if they spend a large amount of time in sedentary behaviours during the rest of the day (Sugiyama *et al.*, 2008).

Increasing sedentary behaviour is becoming a growing problem in the general population (Blair, 2009) and low levels of physical activity are compounded by an increase in physical inactivity. One of the seminal studies linking everyday physical inactivity with adverse health showed that people with jobs that involve a lot of sitting (e.g. bus drivers) had double the incidence of CV disease as those whose jobs include more standing and walking activities (e.g. bus conductors) (Morris *et al.*, 1953). The most direct effect of sitting still is that the work performed by the large skeletal muscles in the legs, back and trunk required for upright movement decreases. Sitting for prolonged periods also causes the loss of opportunity for cumulative energy expenditure resulting from the thousands of intermittent muscular contractions throughout the day (Hamilton *et al.*, 2007). Sedentary behaviours involving sitting or lying down are characterised by a low MET value of less than 2, and lower mean daily MET levels are related adversely to metabolic biomarkers and to poorer health outcomes (Sugiyama *et al.*, 2008).

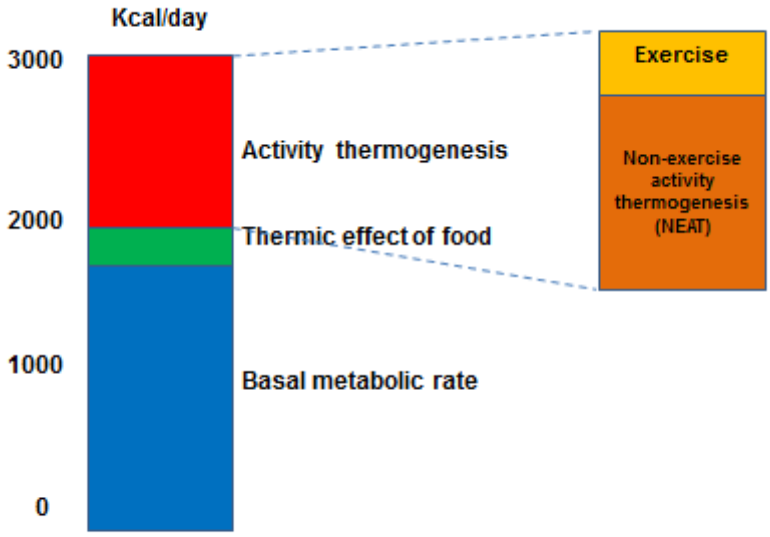
Classically, there are three components of human daily energy expenditure (Figure 3): basal metabolic rate (BMR), the thermic effect of food and activity thermogenesis. BMR is the energy required for the core bodily functions and is measured at complete rest while fasted. It accounts for about 60% of daily energy expenditure in a sedentary person. Nearly all of its variability is accounted for by body size, or more precisely lean body mass, with bigger

people having a higher BMR. The thermic effect of food is the energy expended in response to a meal and is that associated with digestion, absorption and fuel storage. This accounts for about 10% of daily energy needs and does not vary greatly between people. The remaining component, activity thermogenesis can be subdivided into exercise and non-exercise activity thermogenesis (NEAT) which incorporates general, everyday activity. NEAT is the most variable component of human expenditure, and may be the easiest to manipulate for health benefits. NEAT varies between two people of similar size by 2000 kcal/day because of people's different occupations and leisure-time activities (Levine, 2007). Occupations that involve physical labour, such as farming, confer higher NEAT values than those that involve more sedentary work. Variability in leisure also affects NEAT – those people that choose to sit in the evening watching the television exhibit lower NEAT than those that are out walking the dog. Obesity is associated with low NEAT; obese individuals stand and ambulate for 2½ hours/day less than lean sedentary controls (Levine *et al.*, 2005). If we can attempt to address this, either at an individual level by encouraging the person to move more, or at an environmental/societal level by ensuring there are more opportunities to stand/walk throughout the day, then we may have a positive impact on obesity levels and metabolic control.

The links between sedentary behaviour and metabolic health extend beyond the total amount of time spent inactive. Healy et al., (2008a) report that more interruptions in sedentary time were associated with a decrease in metabolic risk factors. This suggests that it is not only the amount of sedentary time that is important, but also the manner in which it is accumulated. As sedentary time comprises a large proportion of waking hours (over 50% for most people - (Hamilton *et al.*, 2007)), small changes regarding the interruption of this with regular, short breaks of light-intensity activity could be incorporated across numerous settings and workplaces, increasing NEAT, resulting in beneficial metabolic effects (Levine, 2007). Regular participation in moderate-vigorous intensity exercise should still be promoted as the predominant physical activity message. However, encouraging a reduction in sedentary time through increasing light-intensity day-to-day activity may be another important public

health message for reducing obesity and overall metabolic risk (Levine, 2007; Healy *et al.*, 2008).

Figure 3: Components of total daily energy expenditure (Levine, 2007)



There is a growing body of evidence reporting that the majority of people at risk of developing the metabolic syndrome, obesity and T2DM, spend excessive amounts of time inactive and have low levels of NEAT (Dunstan *et al.*, 2004; Dunstan *et al.*, 2005; Levine *et al.*, 2005; Healy *et al.*, 2008). These results are real and applicable to our everyday lives, with one study reporting that with every one hour increase of television viewing per day that there was a 26% increase in the prevalence of metabolic syndrome in women (Dunstan *et al.*, 2005). The magnitude of the negative effect of television watching was about the same as the positive health benefit derived from the 30 minutes of extra physical activity/exercise recommended to improve health. Given the balance between the negative health consequences of physical inactivity and the modest positive effects of exercise in comparison, it is important to identify both activity and inactivity in developing clinically meaningful interventions.

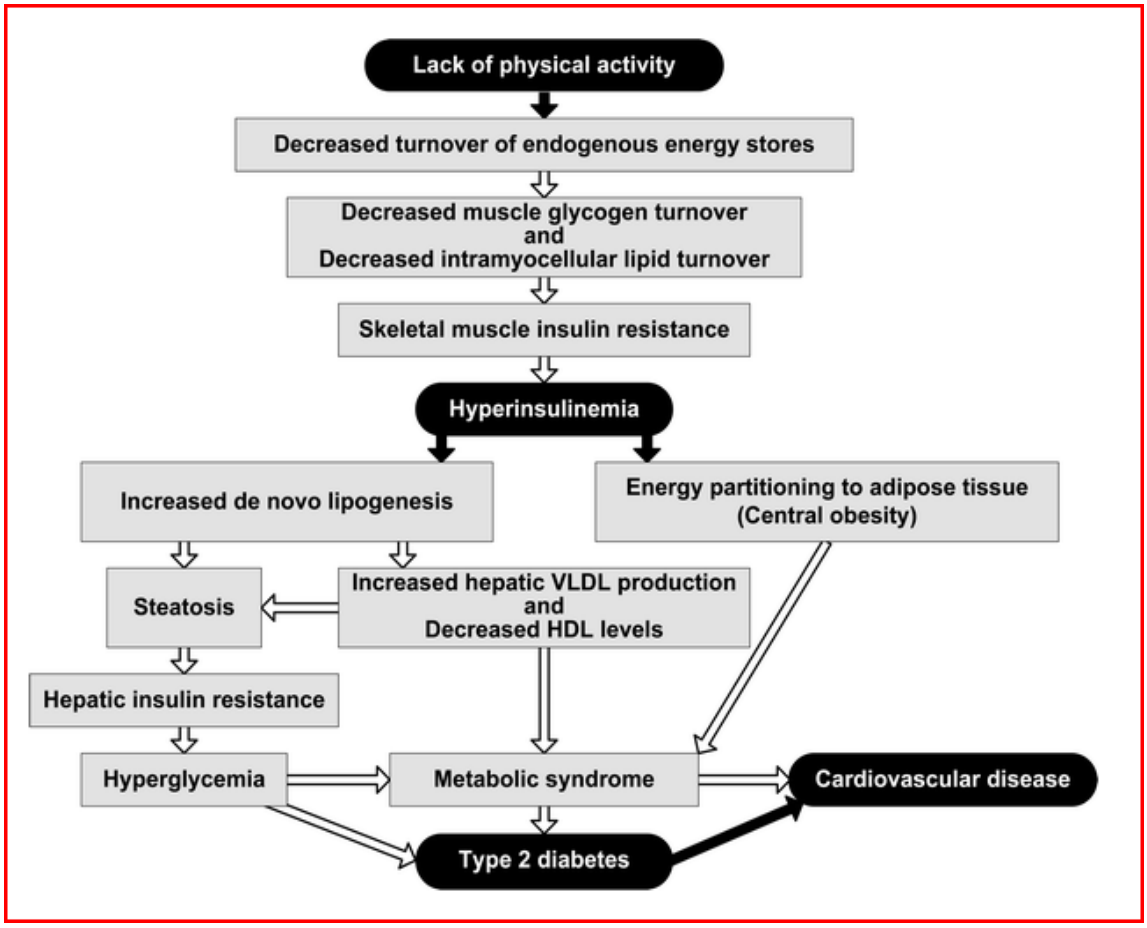
The majority of the general population are unaware of the potential insidious dangers of sitting too much or the possible benefits of at least maintaining daily low-intensity intermittent non-exercise activity throughout the day. Often, these non-exercise activities occur subconsciously. A study using objective and sensitive measures of movement (accelerometry) estimated that sedentary young adults moved their body an equivalent of walking 9 miles per day (Hamilton *et al.*, 2007). Energy expenditure of "standing workers" (e.g. shop assistants) was approximately 1400kcal/day, for work involving some manual labour around 2300kcal/day, whereas seated workers burned only around 700 kcal/day. More than 90% of the calories burned during all forms of physical activity were due to this pattern of standing and non-exercise ambulatory movements (Hamilton *et al.*, 2007). The frequency and cumulative duration of non-exercise activity throughout the day is extremely high. People perform intermittent bouts of non-exercise activity throughout most of the day, 7days/week, 365days/year. In contrast, the frequency of exercise is more limited, generally to less than 150min/week. Given the broader opportunities and implications for daily low-intensity activity, it is possible that maintaining this level of activity has greater implications for health and well-being than moderate-vigorous physical activity for those who do not prefer more structured exercise.

Researchers hypothesise that signals harming the body during high levels of physical inactivity are different from those that boost health above normal after exercising regularly (Hamilton *et al.*, 1998; Bey and Hamilton, 2003). Lipoprotein lipase (LPL) is the first protein directly interacting with and regulating lipoproteins to be studied at the cellular level during physical inactivity. Physical inactivity has a powerful effect on suppressing LPL activity in skeletal muscle, the rate-limiting enzyme for the hydrolysis of triglyceride rich lipoproteins (Zderic and Hamilton, 2006). Local contractile activity and/or inactivity is the major physiological variable regulating LPL function within the skeletal muscle and a localised reduction in contractile activity is a potent physiological factor reducing LPL activity. Low LPL function has been linked

with blunted triglyceride uptake in skeletal muscle and reduced plasma HDL cholesterol levels.

Increased skeletal muscle LPL has been reported following short-term exercise training (Hamilton *et al.*, 1998). LPL activity was measured in 6 muscles after intensive training for 2 weeks. Exercise increased LPL activity 2- to 2.5-fold in the least oxidative regions of the leg muscle (fast-twitch white fibres), whereas, the most oxidative (slow-twitch red fibres) postural leg muscles that already had high LPL due to non-exercise activity, did not display any further increase in LPL after training (Hamilton *et al.*, 2007). LPL activity is generally much greater in the red oxidative muscle types than in the white glycolytic muscles. By removing the normally high level of postural support by oxidative muscles, this abolished the difference of LPL activity between muscle fibre types. This suggests that the difference in LPL activity between fibre types is primarily due to the level of recruitment in normal daily activity (Bey and Hamilton, 2003) and thus, local changes in metabolism during even light-moderate contractions are the most important physiological stimulus for LPL regulation in skeletal muscle.

Figure 4: A schematic representation of the links between physical inactivity with Type 2 diabetes and metabolic disease (Booth *et al.*, 2008)



1.5.2 Physical Inactivity and NAFLD

Increases in sedentary time, could play a potential role in the development of NAFLD and, in turn, provide a potential avenue for therapy. Current physical inactivity physiology would suggest that a reduction in LPL activity, as a result of fewer cumulative muscle contractions throughout the day, could predispose to NAFLD through the resultant circulatory hyperlipidemia. An increase in circulating fatty acids, with fewer being hydrolysed as lipoproteins, will lead to an increased delivery of circulating fatty acids to the liver and hence predisposition to or progression of NAFLD. Increasing circulating fatty acids also exacerbates IR (Taylor, 2008) and hyperinsulinemia which could subsequently increase *de novo* lipogenesis within the liver.

Decreasing overall sedentary time and increasing breaks throughout the day could be a useful therapeutic message to relay to people with NAFLD, and may be perceived as being more achievable by patients initially than increasing physical activity levels. Any means of increasing NEAT, whether it be at work or during leisure time, may exert positive metabolic benefits. To date, no studies have reported sedentary time or NEAT in NAFLD so any relationships remain purely speculative at present.

1.5.3 Physical Activity and Metabolic Control

Public health guidelines promote at least 150min/week of moderate-vigorous leisure-time physical activity to aim at decreasing the risks for metabolic diseases (ACSM, 2009; Department of Health, 2011). However, the majority of people in the general population do not follow this prescription for enough moderate-vigorous exercise and this may be contributing to the rising numbers of people being affected by obesity and T2DM.

Evidence for the benefit of physical activity comes from studies showing that individuals who exercise and maintain a physically active lifestyle are less likely to develop IR, impaired glucose tolerance, or T2DM (Boule *et al.*, 2001; Snowling and Hopkins, 2006; Thomas *et al.*, 2006; Colberg *et al.*, 2010). Physical activity appears to result in insulin-receptor up-regulation in muscle tissue increasing delivery of glucose and insulin to the muscles, and translocation of GLUT4 to the muscle cell membrane, enhancing non-insulin dependent glucose uptake (Hayashi *et al.*, 1997; Zelber-Sagi *et al.*, 2008; Agosti *et al.*, 2009). Exercise also has a beneficial effect on NEFA metabolism by enhancing whole-body lipid oxidation (Trenell *et al.*, 2008; Hallsworth *et al.*, 2011) and favourably affects overall lipid profile (Agosti *et al.*, 2009; Kadoglou *et al.*, 2010), reducing the risk of CV disease. Physical activity, including exercise, has been shown to improve mitochondrial number and density in skeletal muscle (Toledo *et al.*, 2007). This results in an increase in oxidative capacity which enhances fat oxidation. Physical activity offers an insulin independent way of aiding glucose homeostasis in the face of IR and promotes

fat oxidation, thus reducing hyperlipidemia, all of which is key in the prevention and management of metabolic disorders.

1.5.4 Physical Activity and NAFLD

Physical activity levels are reported to be lower in people with NAFLD than their "healthy" counterparts. A cross-sectional study of Japanese men showed that the prevalence of NAFLD (as assessed by echocardiography) was inversely related to the frequency of self-reported exercise (Hsieh *et al.*, 1998). Those people that exercised for more than 30 minutes a day on at least 3 days per week were half as likely to have NAFLD as their sedentary counterparts, despite a similar BMI. In a subsequent cross sectional report, these observations were expanded to state that people without fatty liver engaged in nearly 3 times more resistance activity than people with NAFLD (Zelber-Sagi *et al.*, 2008). Among the NAFLD group, those that engaged in physical activity of any kind or duration had lower fasting serum insulin levels and a lower rate of abdominal obesity even though they had a similar BMI to their inactive counterparts. However, in both of these studies, physical activity levels were obtained from self-reported, non-validated, physical activity questionnaires developed for the purpose of the research, rather than being objectively measured. Perseghin et al. (2007) demonstrated that a higher level of habitual physical activity is associated with a lower level of intrahepatic lipid (IHL) and suggested that this relationship may be due to the effect of exercise per se (n=191). In this study, IHL was assessed via MRS and physical activity via questionnaire (Perseghin *et al.*, 2007a). Again, this study relied upon self-reporting of physical activity levels rather than using an objective measure, but did use a questionnaire validated for use in the general population. Increasing physical activity levels in people with NAFLD is likely to be of benefit, not only to liver health, but the overall metabolic profile, and should be encouraged in a bid to prevent NAFLD progression, the development of T2DM or CV disease.

1.5.5 Physical Activity Measurement

In order to utilise physical activity as a treatment strategy in the management of NAFLD, researchers need a means to accurately measure levels of physical inactivity, physical activity and exercise. These sensitive and specific tools are necessary to best characterise the habitual patterns in these clinical groups and also monitor the effectiveness of interventions. These tools may also assist clinicians in providing accurate feedback to the patient as to their current activity levels, and enable individual activity targets to be set, monitored and worked towards as part of the patient's treatment package. Several different methodologies exist for the measurement and assessment of physical activity and energy expenditure (EE). These methodologies range from expensive and objective laboratory measures such as doubly labelled water to subjective measures such as self-reported physical activity questionnaires. All of these tools have benefits and limitations, and their appropriate use depends on multiple factors which will now be introduced and discussed.

Doubly labelled water: Doubly labelled water (DLW) is the gold standard for measuring total EE during free-living (Bluck, 2008). This method is based on the principle that in a loading dose of $^{2}H_{2}^{18}O$, ^{18}O is eliminated as carbon dioxide and water, while deuterium is eliminated from the body only as water (Lifson *et al.*, 1955). The rate of carbon dioxide production, and thus EE, is calculated from the difference of the two elimination rates. Using the DLW method, subjects are free to carry out their normal day-to-day activities, and EE can be accurately measured over a period of up to 14 days gleaning a good insight into the person's general overall activity levels. Although effective in determining EE over broad periods of time, the DLW technique does not monitor patterns of activity, nor the frequency, duration or intensity of activities. The DLW technique is also expensive to implement, requires specialist equipment and technical expertise limiting its use to specialist centres and small numbers.

Indirect-calorimetry: Indirect-calorimetry measures the oxygen and carbon dioxide that a person inhales and exhales, and from this, indirectly computes

the calories burned during the period of assessment (Frayn, 1983). This method is normally carried out under laboratory conditions and is considered a good estimate of EE, with an error rate of 3-10% compared to DLW (Wells and Fuller, 1998). Disadvantages of this method are the expensive and bulky equipment, and the use of the mask for gas analysis which limits the type of activities that can be studied and the duration of measurement.

Physical activity questionnaires: There are a large number of self-recall physical activity questionnaires. The most frequently used are the Baecke and IPAQ. These demonstrate reasonably good agreement with DLW in determining energy expenditure (Philippaerts *et al.*, 1999; Hagströmer *et al.*, 2006; Maddison *et al.*, 2007). Self-reported physical activity is valid (Craig *et al.*, 2003; Hagströmer *et al.*, 2006; Bull *et al.*, 2009) and useful in understanding broad differences in physical activity in large cross-sectional studies. However, these techniques are not sensitive to monitor changes in activity patterns or allow accurate determination of EE and are subject to recall error (Warren *et al.*, 2010). Differences between self-report and DLW may be as high as 30% (Irwin *et al.*, 2001).

Heart rate monitors: Heart rate monitors are routinely used to measure physical activity in both research and recreation, with an increase in heart rate used as a surrogate marker for an increase in physical exertion. However, heart rate monitors are only accurate in measuring moderate-vigorous activities, as in lower intensity activities, confounding factors, such as stress, emotions, illness and caffeine intake, have a significant impact on results (Crouter *et al.*, 2004). Heart rate monitors may therefore be deemed an inappropriate technique, when used in isolation, for measuring day-to-day activity which is generally of low-moderate intensity.

Pedometers: Pedometers are simple devices, which use up and down motions as estimates of steps. Pedometers provide a low cost means of crudely

measuring physical activity. The major drawback to this method is that pedometers measure footfalls, and thus any activity undertaken which doesn't involve ambulation (e.g. weight lifting, biking, swimming), is inaccurately recorded. Pedometers also fail to capture intensity, frequency or duration of activity. In most cases, pedometers prove accurate in counting steps, however, they are much less accurate in predicting EE, with error rates of ±30% (Crouter *et al.*, 2003).

Accelerometry: An accelerometer is an electromechanical device that will measure acceleration forces. Basic, uniaxial accelerometers measure acceleration of the body or body parts in one plane and take into account the speed, direction and duration of movements and convert these to movement counts to allow for estimation of EE. Biaxial or triaxial accelerometers provide information about movement in multiple directions, and show a better relationship to physical activity EE than uniaxial units (Plasqui and Westerterp, 2007). All accelerometers are subject to motion artefacts, and cannot distinguish movement from activities such as driving a car, from actual "physical" activity. Error rate for accelerometry ranges from 14-30% against laboratory measures (Fehling *et al.*, 1999; Chen *et al.*, 2003) with uniaxial units prone to the greatest recording error due to their relative insensitivity to whole body movement.

Multi-sensor array: Multi-sensor systems, or multi-sensor arrays, combine measures such as heart rate, accelerometry and body temperature to provide an overall more accurate picture of physical activity patterns. Multi-sensor arrays utilise pattern detection algorithms (typically determined by the respective manufacturer) to combine physiological signals detected from the different sensors to first identify the wearer's context, and then apply an appropriate formula to estimate EE from the sensor values (Welk *et al.*, 2000). These monitors are generally easy and comfortable to use and have an average error rate of 8-10% when compared to laboratory measures (Welk *et al.*, 2000; St-Onge *et al.*, 2007).

1.5.6 Exercise and Metabolic Disease

Exercise alone (see Section 1.5 for definition), in the absence of any change in body weight or composition, may enhance insulin sensitivity and glucose homeostasis. Exercise, or muscle contraction per se, provides an insulin independent way of stimulating glucose uptake from the circulation into skeletal muscle. As the muscle contracts, GLUT4 transporters translocate to the muscle cell wall increasing the capacity for glucose uptake (Zelber-Sagi *et al.*, 2008). A larger mass of skeletal muscle, as a consequence of exercise, increases overall glucose storage capacity. Exercise also enhances fatty acid metabolism by enhancing whole-body lipid oxidation (Trenell *et al.*, 2008; Hallsworth *et al.*, 2011). Thus, in people who are IR or have T2DM, exercise provides a way of improving glycaemic control.

A meta-analysis looking at the effects of different modes of exercise training on glucose control concluded that aerobic, resistance, and combined exercise have small to moderate beneficial effects on glucose control in patients with T2DM. The reduction in HbA1c achieved with exercise was similar to that with long-term drug or insulin therapy (Snowling and Hopkins, 2006). The effect of duration of the exercise programme on HbA1c was consistent with the turnover time for haemoglobin and red blood cells (i.e. greater than 8 weeks), but otherwise, the effect of total exercise time on HbA1c was not linked. This finding is consistent with most patients reaching a stable state in their exercise programmes and gaining no extra benefit from more exercise. The meta-analysis also found that there may be little difference in the effectiveness of programmes differing in intensity. This meta-analysis included 27 controlled trials using supervised exercise training programmes of patients with T2DM.

Another meta-analysis looked at the effects of exercise on glycaemic control in patients with T2DM (Boule *et al.*, 2001). They included 14 controlled clinical trials that had an exercise component which lasted for at least 8 weeks. They found that when the post-intervention results were pooled, HbA1c was significantly lower in the exercise groups compared with the control groups. Exercise training was found to reduce HbA1c by approximately 0.66%, an

amount that would be expected to reduce the risk of diabetic complications significantly. However, the analysis included some trials where diet was used as a co-intervention with exercise in the intervention group, making it difficult to measure the effects of exercise independently. Further subgroup analysis comparing aerobic or resistance training groups revealed no significant difference. Exercise intensity and volume were not associated with the post-intervention difference in HbA1c.

A recent Cochrane Review (Thomas *et al.*, 2006) looking at exercise as an intervention for T2DM, found that exercise significantly improved glycaemic control and reduced VAT and plasma triglycerides in people with T2DM, even without weight loss. Fourteen controlled clinical trials were included in the review comparing exercise to no exercise in patients with T2DM. HbA1c was reduced by 0.6% with exercise and this decrease was found to be more pronounced in the shorter studies (studies of 3 months or less). This probably reflects both the higher intensity of exercise in some of the shorter trials, as well as the difficulties of maintaining compliance with exercise programmes in longer term studies. The mean reduction in HbA1c compares well with reported reductions achieved through diabetes medications such as metformin (Johansen, 1999).

In patients with T2DM, skeletal muscle mitochondria are reduced in size, and there is reduced activity of the electron transport chain (Petersen *et al.*, 2004). Mitochondria are normally adaptable organelles and in skeletal muscle in healthy individuals there is considerable plasticity in terms of mitochondrial content, allowing the muscle to adapt to match energy demands of physical activity (Toledo *et al.*, 2007). Endurance training increases fat oxidation during submaximal exercise. Mild or moderate intensity exercise (25-65% of VO_2max) is associated with a 5-10 fold increase in fat oxidation above resting amounts because of increased energy requirements of muscle and enhanced fatty acid availability (Horowitz and Klein, 2000). Several factors contribute to this adaptive response: increased density of the mitochondria in the skeletal muscles, which increases the capacity for fat oxidation; a proliferation of

capillaries within skeletal muscle, which enhances fatty acid delivery to muscle; an increase in carnitine transferase , which facilitates fatty acid transport across the mitochondrial membrane; and an increase in fatty acid binding proteins, which regulate myocyte fatty acid transport (Horowitz and Klein, 2000; Goodpaster *et al.*, 2003). In people with T2DM, mitochondria were found to increase both in size and density after a 4-month lifestyle intervention of daily moderate-intensity exercise with moderate weight loss (Toledo *et al.*, 2007). Increased fatty acid oxidation during endurance exercise permits sustained physical activity and delays the onset of glycogen depletion and hypoglycaemia.

Compared with untrained persons exercising at the same absolute intensity, people who have undergone endurance training have greater fat oxidation during exercise without increased lipolysis. Available evidence suggests that the training-induced increase in fat oxidation is due primarily to increased oxidation of non-plasma-derived fatty acids, perhaps from intramuscular triglyceride stores (Horowitz and Klein, 2000). Several studies suggest that intramuscular triglycerides represent a considerable portion of the total fat used during endurance exercise and may provide over 50% of the total fat oxidised during exercise (Horowitz and Klein, 2000). A decrease in intramuscular fat stores would improve insulin sensitivity of the muscles and enhance overall glycaemic control.

1.5.7 Resistance Exercise and Metabolism

Resistance exercise, often known as strength or weight training, works the muscles against a load. Resistance exercise provides an alternative to aerobic exercise; it improves muscular strength, muscle mass and metabolic control, safely and effectively, in vulnerable populations independent of weight loss (Larose *et al.*, 2010). It places less of a demand on the cardio-respiratory system and may therefore be accessible to more patients (Gordon *et al.*, 2009).

Evidence that resistance exercise can improve body composition is increasing and it is now recommended by the American College of Sports Medicine and

the American Heart Association as an integral component to any exercise programme (Ormsbee *et al.*, 2007; Ormsbee *et al.*, 2009). A meta-analysis comparing aerobic training with weight training concluded that weight training resulted in greater increases in fat-free mass (Ballor and Keesey, 1991). An increase in muscle mass may improve insulin sensitivity by increasing the available glucose storage area, thereby reducing the amount of insulin required to maintain a normal glucose tolerance. An increased muscle mass may also improve fat oxidation due to an increase in the number of mitochondria.

Resistance exercise has been shown to decrease respiratory exchange ratio (RER) after exercise, indicating elevated fat oxidation (Ormsbee *et al.*, 2009). This reduction in RER has been reported to last hours after a single bout of resistance exercise (Melby *et al.*, 1993; Ormsbee *et al.*, 2007). This represents a shift toward greater fat relative to carbohydrate oxidation during the post-exercise period. Enhanced fat oxidation, observed as an acute response to resistance exercise, is due to glucose sparing for the purpose of glycogen replenishment, thus resulting in fatty acids being the primary substrate for energy provision after resistance exercise.

Strenuous resistance exercise could be beneficial in weight control, not only because of the direct caloric cost of the activity and the residual elevation of the post-exercise VO_2 but also because of the greater post-exercise fat oxidation. Energy expenditure has been found to be elevated for as long as 38 hours after an acute bout of heavy resistance exercise (Schuenke *et al.*, 2002). Results suggest that the energy required to recover from resistance training may be of significant use to a weight control/loss programme. For the first 24 hour period following exercise, metabolism was increased by 21.2% and over a further 24 hours by 19.3%. These differences could equate to 404kcal and 369kcal increases per day, respectively for average build individuals (Schuenke *et al.*, 2002).

After exercise, oxygen uptake remains elevated above resting levels for a period of time. This sustained post-exercise elevation in oxygen uptake has been referred to as excess post-exercise oxygen consumption (EPOC), previously known as the oxygen debt. In the case of resistance exercise, it appears that the intensity of the exercise is very influential in determining the duration of EPOC (Schuenke *et al.*, 2002). Data suggests that exercise intensity has a greater impact on the magnitude of EPOC than does exercise duration. High intensity resistance exercise of longer duration may result in a prolonged recovery period, contributing to significant post-exercise caloric expenditure (Melby *et al.*, 1993) but may not be achievable by the majority of the general population. Studies have found that there is no sustained elevation of metabolic rate after exercise of intensities <55% VO_2max and <3h duration (Borsheim *et al.*, 1998). Thus the low- to moderate-intensity exercise, capable of being performed by the general public, produces little excess energy expenditure during recovery and would appear to have little impact on weight control. The benefit of such exercise in terms of caloric expenditure is limited almost entirely to the exercise period itself.

The lipolytic response in adipocytes is a function of the interplay between the opposing effects of the stimulatory β-adrenergic receptors (β-ARs) and the inhibitory α-adrenergic receptors (α-ARs) that are expressed in human fat cells. Catecholamines (adrenalin, noradrenalin and dopamine) are the only hormones with a marked lipolytic effect on the fat cells of humans and regulate lipolysis in adipose tissue by interacting with both the α-ARs and β-ARs. Catecholamines stimulate lipolysis through β-ARs. Studies using isolated human adipocytes show that β-adrenergic stimulation of lipolysis is weakened by activation of α-ARs by adrenalin and noradrenalin. α-adrenergic inhibiting effects have been found to modulate lipolysis at rest, while β-adrenergic stimulating effects are important to modulate lipolysis during exercise (Arner *et al.*, 1990; Borsheim *et al.*, 1998).

The anti-lipolytic α-ARs predominate in subcutaneous abdominal adipose tissue and adrenalin has a higher affinity for α-ARs than β-ARs. Adrenalin and

noradrenalin levels were found to be significantly increased immediately after exercise and a rise in catecholamines likely contributed to the increased lipolysis (Ormsbee *et al.*, 2007). Resistance exercise can also lower intramuscular lipids in skeletal muscle presumably by activating lipolysis. Like lipolysis in subcutaneous adipose tissue, catecholamines can activate lipolysis in the intramuscular lipid stores. Dynamic strength training over a 3-month period was able to increase lipolysis in obese men by more efficiently stimulating β-ARs. Therefore, chronic or acute resistance exercise may help to prevent weight gain and improve body composition through the mechanisms of increasing energy expenditure, abdominal subcutaneous lipolysis and whole body fat oxidation (Ormsbee *et al.*, 2007). Pharmacologically blocking α-ARs with phentolamine has been reported to elevate aerobic exercise-induced lipolysis, especially in obese subjects (Ormsbee *et al.*, 2009). Blocking β-ARs with propranolol after exercise was found to reduce EPOC (Borsheim *et al.*, 1998).

Catecholamines are much more lipolytic in the abdominal than in the gluteal/femoral fat depots indicating that lipids are mobilised more readily from the abdominal region during exercise (Arner *et al.*, 1990). Encouraging exercise, may help to increase lipolysis in the more pathogenic abdominal fat stores resulting in added health benefits.

1.5.8 Exercise and NAFLD

In NAFLD, increased physical activity and exercise are widely recommended in disease management, however, the independent effect of exercise on IHL and liver enzymes is hard to determine as most studies have used combined exercise and diet interventions with and without weight loss. Weight loss remains the most common therapy promoted for reducing IHL in NAFLD, although diet-only induced weight loss is often not sustainable and current research is looking to determine the effects of exercise as an independent treatment modality (Johnson *et al.*, 2009; Hallsworth *et al.*, 2011). People with NAFLD are encouraged to increase their physical activity and exercise levels,

because studies in people without this condition have found that exercise may reduce hyperglycaemia and body fat and improve protection against developing CV complications. Even though exercise is recommended as part of treatment for NAFLD, there have been no large-scale studies with adequate statistical power to guide health practitioners in prescribing exercise programmes for the management of these patients. The optimal type, frequency, intensity and duration of exercise for achieving therapeutic goals in NAFLD are unknown.

Table 1: Summary of exercise only intervention studies in adults with NAFLD

Reference	Design	Sample Size	Clinical Group	Age (yrs)	BMI (kg/m^2)	Intervention	Duration (weeks)	Outcome Measures	Results
(Hallsworth *et al.*, 2011)	CT	11 INT 8 CON	NAFLD	52±4 62±3	32±2 32±2	INT: 3 unsupervised resistance training sessions/wk CON: Standard care (no exercise)	8 8	IHL, fsOGTT, HOMA-IR, fat oxidation	IHL↓13%, 12%↑in insulin sensitivity, ↑ fat oxidation during exercise, ↓HOMA-IR in INT group; no changes in CON group
(Johnson *et al.*, 2009)	RCT	12 INT 7 CON	NAFLD	47±4 49±2	31±1 32±2	INT: 3 supervised cycle ergometer sessions/wk CON: 3 home-based whole body stretching sessions/wk	4	IHL, ALT, HOMA	IHL↓21% in INT group; no change in ALT. No change in HOMA, fasting glucose or insulin within or between groups
(Sreenivasa Baba *et al.*, 2006)	UCT	16	Elevated ALT	37±2	23±0	45mins/6 d/wk. Unsupervised. Exercise included walking, jogging and rhythmic aerobic exercises	12	ALT and AST	From baseline: ALT↓47%, AST↓48%

CT, controlled trial; RCT, randomised controlled trial; UCT, uncontrolled trial; INT, intervention; CON, control

The effect of aerobic exercise on IHL, independent of weight loss, has not been clarified. Johnson et al. (2009) found that four weeks of aerobic exercise, three times per week, significantly reduced VAT by 12% and IHL by 21%. In absolute terms, IHL decreased from 8.6 to 6.8%. This was associated with a 14% reduction in plasma NEFA. Exercise training did not alter body weight, vastus lateralis intra-myocellular triglyceride concentration, abdominal SAT or homeostasis model assessment of insulin resistance (HOMA-IR) (Johnson *et*

al., 2009). This may have been due to the short duration of the exercise intervention. This study concluded that regular aerobic exercise reduces IHL in obesity even in the absence of body weight reduction and thus that aerobic exercise should be promoted for the management of NAFLD. However, the authors did not investigate what happened after the four-week exercise period, and how long these benefits were sustained with/without continuation of the regular exercise programme. A further study using a 12-week (four times per week) aerobic exercise programme without weight loss, was found to reduce accumulation of IHL by 37% in a group of 15 obese adolescents (van der Heijden *et al.*, 2009). In absolute terms, IHL decreased from 8.9 to 5.6%. IR also improved and VAT was reduced although subcutaneous and intra-myocellular fat content remained unchanged. Results from this study cannot be generalised to adults with NAFLD as the mechanisms responsible for the changes may prove to be different in adolescents.

Devries et al. (2008) looked at the effect of endurance exercise training on IHL and liver enzymes in men and women without weight loss. Subjects underwent a 3-month aerobic training programme 2-3 times per week at a moderate intensity. They found that there was no effect of endurance training on IHL or IR. They also found that there was no positive influence of endurance exercise on hepatic liver enzymes. This could be related to the relatively short duration of the study or the fact that the patients had baseline liver enzymes (ALT and GGT) levels within normal physiological ranges. This study was also designed to prevent weight loss and exercise-induced weight reduction appears to be more strongly linked to improvements in liver fat and liver enzymes (Devries *et al.*, 2008). The study did show that a 12-week endurance training programme did have positive effects on abdominal obesity and aerobic capacity.

Krasnoff et al., (2008) found that increasing NAFLD severity was linked to decreasing cardiorespiratory fitness. They also found that irrespective of the severity of NAFLD, patients had suboptimal cardiorespiratory fitness, muscle strength, body composition, and physical activity participation. In their study, less than 20% of the patients with NAFLD reported participating in regular

physical activity or exercise and approximately 20% reported participating in absolutely no leisure-time physical activity at all (Krasnoff *et al.*, 2008). A further study found that there was a lower prevalence of NAFLD in people with higher levels of cardiorespiratory fitness and a higher level of NAFLD in people with a higher BMI and waist circumference, and lower fitness (Church *et al.*, 2006). Patients with a higher fitness level at baseline, prior to engaging in a lifestyle intervention, have been shown to achieve greater improvements in IHL and even a resolution of NAFLD (Kantartzis *et al.*, 2008). This study suggests that measurement of fitness could be useful in identifying patients with NAFLD who are more likely to respond to an exercise intervention in isolation, or those who may require supplementary input in terms of either diet or drug therapy.

St. George et al. (2009) investigated the effects of changes in physical activity on the metabolic profile of patients with NAFLD. They assessed the impact of a behaviour change-based lifestyle intervention after 3-months in this patient group and found that those patients increasing or maintaining their physical activity to at least 150 minutes/week, and those that increased their objective levels of fitness had the greatest improvements in liver enzymes and other metabolic indices compared with those that were least active. This effect was independent of weight loss and was corroborated by an objective measure of fitness. The study also found that there was no dose-response effect on liver enzymes with incremental increases in physical activity above 60 minutes/week (St. George *et al.*, 2009). Among patients with NAFLD, small increases in regular physical activity, even in the absence of weight loss, can contribute to improvements in liver enzymes and thus small gains in fitness may have significant health benefits for patients with NAFLD.

Systemically, exercise improves whole-body fat oxidation in adipose, intra-myocellular (Ormsbee *et al.*, 2007), and possibly hepatic tissues leading to a decrease in circulating fatty acids. It also leads to a proliferation of capillaries within skeletal muscles thus delivering fatty acids to the muscle cells more efficiently. Within the muscle cells there is an increased density of mitochondria, and an increased transfer of fatty acids into the mitochondria. An exercise-

induced increase in mitochondrial enzyme content and higher numbers of fatty acid binding proteins enhance the oxidation of fatty acids. There is a significant increase during and after exercise in vLDL secretion and vLDL clearance by skeletal muscle, which may accelerate the clearance of fatty acids derived in the liver. Exercise also helps to reduce abdominal and visceral fat which are both main sources of the fatty acids that are released into the plasma which then become available for uptake by the liver.

VAT is currently believed to be the key depot linked with obesity-related systemic metabolic disturbances. Mobilised fatty acids produced from the lipolysis of VAT are directly trafficked through the liver via portal circulation making it no surprise that increased visceral adiposity is strongly correlated to fatty liver (Church *et al.*, 2006). VAT becomes inflamed during adipose tissue hypertrophy due to an influx of macrophages that secrete proinflammatory cytokines, including TNFα. Reducing inflammation in VAT beneficially modifies these metabolic disturbances and decreases disease risk, even in the absence of obesity reduction. Exercise training has been shown to decrease general chronic low-level systemic inflammation in humans as well as improve IR and hepatic steatosis (Vieira *et al.*, 2009). Unfortunately, the direct effects of exercise, with or without dietary changes, on VAT inflammation has not been adequately assessed in humans. However, animal models have shown that exercise training lowers VAT inflammation in the viscera of non-obese animals suggesting that exercise may be a useful therapy (Vieira *et al.*, 2009).

Vieira et al (2009) looked at the effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice. They found that moderate exercise, low fat diet, or their combination resulted in decreases in systemic and VAT inflammation. These effects were stronger after 12 weeks than 6 weeks. Combining the exercise with dietary modification had more of an effect than either intervention on its own. Whilst the low fat diet induced an initial greater amount of weight loss, exercise was more effective at preventing adipose tissue accumulation in the viscera. Both treatments were found to reduce hepatic steatosis at 6 weeks, but only exercise did so by 12 weeks.

Previous studies have shown that weight loss and exercise reduce hepatic steatosis, whereas high-carbohydrate diets (such as the low fat diet used in this study, which was 70% sucrose) may increase hepatic steatosis by promoting *de novo* lipogenesis. Importantly, Vieira et al (2009) showed that exercise, regardless of diet, is an effective means to prevent hepatic steatosis.

The finding that exercise has unique anti-inflammatory effects is novel and likely will have important implications, given the strong independent relationship between VAT inflammation and obesity-related comorbidities and the lack of long-term success of dietary interventions without exercise. Mechanisms by which exercise reduces VAT inflammation may involve improvements in the "health" of the VAT, including reduced adipose size, increased blood flow, increased mitochondrial function and facilitated fatty acid oxidation, decreased cellular stress, and/or improved resistance to cell stress (Vieira *et al.*, 2009). Recent studies have implicated hypoxia as a cause of adipose tissue inflammation in obesity – an exercise-related increase in VAT blood flow may mediate its anti-inflammatory effects on VAT.

1.5.9 Lifestyle Interventions (Diet plus Exercise) and NAFLD

IR and obesity (particularly abdominal obesity) represent the most important risk factors for the development of NAFLD. Because lifestyle modification, including weight reduction and physical activity, has been shown to reduce many of the risk factors for NAFLD, it has become the primary treatment modality (Zelber-Sagi *et al.*, 2008). Furthermore, lack of exercise, which can have a profound effect on skeletal muscle lipid turnover, is indicated in this lipid-induced IR.

Although it is known that caloric restriction, weight loss, and exercise training improve insulin sensitivity, the extent to which these interventions influence IHL accumulation has not been adequately explored. One study (Larson-Meyer *et al.*, 2008) found that caloric restriction, with or without exercise, is an effective lifestyle modification that simultaneously reduces IHL and improves the metabolic profile. Another study (Ueno *et al.*, 1997) used a 3-month restricted diet and exercise intervention in a group of obese patients with NAFLD (15

intervention; 10 control) and found this to have beneficial effects on BMI, liver enzymes, total cholesterol, glucose and the degree of hepatic steatosis.

Shah et al. (2009) compared a 6-month diet (D) only and a diet and exercise (D+E) intervention in a group of obese older (65-82 years) adults to look at IHL and insulin sensitivity. They found that weight loss caused by D or D+E was equally effective in reducing IHL (by ~50%) and improving insulin sensitivity (by ~60%). This 6-month intervention produced a marked reduction (by ~70%) in the prevalence of NAFLD in both groups. This study found that exercise training did not have an additive effect on diet-induced weight loss in reducing IHL. The authors suggested that exercise is a possible preventative approach to NAFLD management but may not be an effective treatment without weight loss (Shah *et al.*, 2009).

Weight loss through caloric restriction is known to improve and even reverse IR and adding an endurance exercise training programme to a calorie restricted diet has been proposed to enhance improvements in insulin sensitivity (Schenk *et al.*, 2009). However, differentiating the effects of exercise training from the effects of weight loss is very difficult. Weight loss (i.e. a reduction in fat mass) can reduce systemic fatty acid mobilisation, which itself can improve insulin sensitivity. Exercise training increases fatty acid oxidation, although exercise training without weight loss fails to improve insulin sensitivity. This may be explained by the fact that exercise training without weight loss does not reduce fatty acid mobilisation and uptake.

Schenk et al. (2009) compared a weight loss programme with weight loss plus exercise. They found that fatty acid mobilisation and uptake were more than 30% lower in both groups after weight loss. However, consistent with an increase in maximal oxidative capacity, resting whole-body fatty acid oxidation was 20% higher after weight loss plus exercise. This increase was due to a greater contribution of fatty acids to total energy expenditure (i.e. decreased RER). Another study found that a 10-week diet plus exercise and an exercise-

only intervention both improved anthropometric measures, insulin sensitivity, liver ultrasound findings and physical fitness in patients with NAFLD. However, improvements were greatest in the diet plus exercise group (Chen *et al.*, 2008).

Behaviour therapy has been designed to provide patients with a set of principles and techniques to modify their eating and activity habits. Appropriate counselling programmes have been found to be of benefit in patients with NAFLD/NASH although these need to be delivered by individuals trained to carry out lifestyle modification interventions (Bellentani *et al.*, 2008). Although most physicians are well aware of the healthy dietary and exercise guidelines to be suggested, few receive adequate training to establish effective communication to promote lifestyle change. Bellentani et al. (2008) recommended that by increasing diet structure and limiting food choices, adherence could be improved. However, the less structure the physical activity component had the better. Self-monitoring improved compliance with both diet and physical activity and favoured weight loss.

A study in Japan evaluated a 6-month home-based lifestyle modification intervention in 67 patients with NAFLD, however, only 22 of these completed the study (the majority of people withdrew due to work commitments). The aim of this intervention was a weight loss of 5% of the initial body weight within the 6 month period. This was achieved via a combination of nutritional counselling and exercise therapy, with all patients continuing in a free-living environment. The intervention resulted in clinically relevant improvements in body weight, VAT, IHL, ALT levels and IR (Oza *et al.*, 2009) but the high drop-out rate of participants must be taken into consideration.

1.6 Cardiac Function in Metabolic Disease

Inadequate metabolic control has a profound impact upon cardiovascular (CV) health. People with T2DM are up to 5 times (Garcia *et al.*, 1974) and people with NAFLD twice (Ekstedt *et al.*, 2006) as likely to have CV disease than people with good metabolic control. A number of studies have described structural and functional alterations in the hearts of people with T2DM and insulin resistance. These adaptations are particularly important as CV disease is the leading cause of death in people with T2DM (Chopra and Peter, 2012). Diabetic cardiomyopathy, defined as cardiac dysfunction in the absence of CV disease (Aneja *et al.*, 2008), is frequently under-diagnosed and may in part account for the high prevalence of cardiac related mortality. The aetiology of the disease includes altered myocardial metabolism and a potential metabolic inflexibility linked to hyperglycaemia and hyperlipidemia (Larsen and Aasum, 2008). Alterations in cardiac metabolism appear to be fundamental to these changes and contributes to cardiac diastolic dysfunction and occasionally progresses to systolic dysfunction leading to heart failure. In the Framingham Heart Study, women with T2DM were found to have higher left ventricular (LV) mass corrected for height and thicker LV walls; women with impaired glucose tolerance showed similar less marked changes. Men with T2DM exhibited a decrease in longitudinal shortening but not change in LV mass or wall thicknesses (Galderisi *et al.*, 1991). Echocardiography demonstrated higher LV mass, increased wall thicknesses, and lower LV longitudinal shortening in 1810 people with T2DM, compared with 944 healthy controls (Devereux *et al.*, 2000). However, this study was undertaken in American Indians so results may not be generalisable to other ethnic groups.

In light of the close links between obesity and NAFLD, early observations in obesity began to highlight cardiac function as a possible therapeutic target. One of the first reports associating obesity with cardiac dysfunction arose from the Framingham Heart Study. This report identified that LV mass was correlated with body mass index (BMI) ($p<0.01$) in nearly 4000 people without a clinical diagnosis of CV disease (Lauer *et al.*, 1991). The relationship between LV mass

and BMI was corroborated in a larger cohort (n=5,098) also including participants free of overt CV disease (Turkbey *et al.*, 2010). Interestingly, ejection fraction, a marker of systolic function, was not associated with BMI (p=0.80). The increase in LV mass was attributed to increases in LV wall thickness and LV internal dimension. Similar findings were made in other studies of otherwise healthy, overweight subjects, data demonstrating a correlation between BMI, LV mass and wall thickness, whereas ejection fraction remained normal (Peterson *et al.*, 2004; Wong *et al.*, 2004; Powell *et al.*, 2006). A number of studies have now reported diastolic dysfunction in people that are overweight or obese. A higher BMI was found to be associated with impaired LV relaxation and elevated LV diastolic filling pressures in a study of obese patients without coronary artery disease (Powell *et al.*, 2006). Another study of hypertensive patients, found that those with high blood pressure and ultrasound diagnosed NAFLD had a higher prevalence of LV diastolic dysfunction than those with hypertension without fatty liver (62.5 vs. 21.1%; p<0.001) (Fallo *et al.*, 2009). LV mass was bigger in those with fatty livers than those without (196.9 ± 75.2 vs. 170.8 ± 62.1g; p=0.07), but this only reached significance in those with more severe liver disease. Measures of cardiac structure and function were made using echocardiography. Since the broad associations between BMI and cardiac function have been reported, attention has been paid to which elements of obesity result in these cardiac adaptations, with a considerable focus on metabolic health.

As NAFLD is closely related to both BMI and metabolic control, it is not surprising that liver health has been included in these studies. An early study of cardiac health in people with NAFLD reported alterations in cardiac morphology and energetics in young men with newly-diagnosed NAFLD using magnetic resonance techniques (Perseghin *et al.*, 2008). Cardiac morphology was not different in those with NAFLD compared to those without fatty liver, however, cardiac metabolism (assessed using ^{31}P-magnetic resonance spectroscopy) was significantly different in the NAFLD group, demonstrated by a decrease in PCr to ATP ratio. The authors suggested that in NAFLD, abnormalities in cardiac metabolism might precede the development of functional and structural

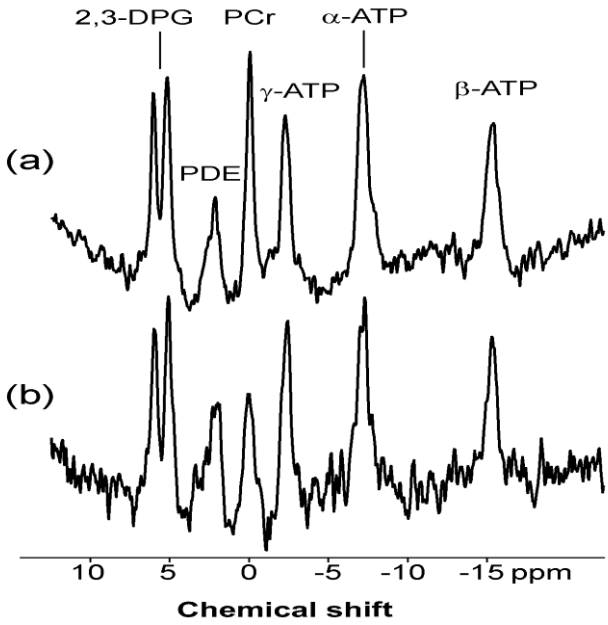
re-modelling of the heart. However, it is difficult to generalise the results of this study to the usual NAFLD population as this only included young (mean age 35), normotensive, non-obese (mean BMI 27.5) males and excluded people who had other endocrine/metabolic disease. A retrospective review of patient records for the presence of significant CV disease (stroke, angina, myocardial infarction, congestive heart failure or the need for revascularisation) was conducted in 219 patients with biopsy-confirmed NAFLD, including NASH and non-NASH fatty liver (Domanski *et al.*, 2012). The overall prevalence of CV disease was 7% and, after controlling for confounders, there was no increased risk of CV disease in those patients with NASH compared with non-NASH fatty liver. However, it should be noted that these sample sizes are small and large prospective studies are lacking in this field. Irrespective of this, given the central role of the liver in metabolic control, it is not surprising that the key mediating effects of the liver upon cardiac health appears to be through its influence in metabolic control, although the independent influence of NAFLD upon CV disease remains an area of discussion.

The heart consumes more energy than any other organ and works continuously to pump blood around the body. To acquire the energy that is needed to carry out this function, the heart converts chemical energy stored in fatty acids and glucose into the mechanical energy utilised by the muscle fibres to make the heart contract. A healthy heart is a metabolically flexible organ, using whatever substrate is freely available as fuel. Normally, NEFA, glucose, and lactate are metabolized for ATP production in the myocardial mitochondria with fatty acids being the preferred substrate, supplying up to 70% of ATP. Generally, NEFA are the primary fuel for the heart in the fasting state and glucose in the postprandial period. The persistent hyperlipidemia seen in T2DM, alters the substrate available to the heart and affects its metabolism. In patients with T2DM, the contribution of glucose oxidation to cardiac energetics is less than normal and the reliance on fatty acid oxidation is increased as high concentrations of NEFA inhibit glucose utilization (Larsen and Aasum, 2008). As a result of this decrease in glucose uptake, cardiomyocytes are faced with a decreased glucose oxidation rate and a marked increase in fatty acid oxidation

– up to 100% of ATP production. This increases the oxygen requirement per ATP molecule produced and reduces cardiac efficiency, rendering the heart more susceptible to energy depletion under conditions of reduced oxygen delivery or increased workload (Scheuermann-Freestone *et al.*, 2003; Carley and Severson, 2005). Although magnetic resonance techniques provide useful information about cardiac morphology and metabolic efficiency, they are limited in their ability to characterise dynamic uptake of substrates, which ultimately underpin some of these adaptations.

The alterations in cardiac metabolism result in impairments in cardiac metabolic efficiency. ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) permits evaluation of myocardial bioenergetics, and hence metabolic efficiency, by calculation of the PCr/ATP ratio (Crilley *et al.*, 2003). This is calculated from the ratio of the PCr signal peak area to the γ-ATP signal peak area – see Figure 5. The PCr/ATP ratio is reduced in systolic dysfunction and in hypertrophic cardiomyopathy (HCM) with normal systolic function (Neubauer *et al.*, 1992). Cardiac energetics have been shown to be significantly impaired in cardiac muscle in people with T2DM who had apparently normal cardiac morphology and function (Diamant *et al.*, 2003; Scheuermann-Freestone *et al.*, 2003). A ^{31}P-MRS study of patients with dilated cardiomyopathy has shown a low PCr/ATP ratio to be a strong predictor of total and CV mortality, superior to the measurement of ejection fraction (Neubauer *et al.*, 1997). Scheuermann-Freestone et al (2003) found that the myocardial PCr/ATP ratio was 35% lower in people with T2DM, who had normal cardiac function, than in healthy control subjects. The PCr/ATP ratio correlated negatively with the plasma NEFA concentrations in all subjects and positively with fasting glucose concentrations in diabetic patients. Abnormal cardiac metabolism has also been demonstrated in obese men, without any other changes in cardiac structure or function (Perseghin *et al.*, 2007b), where BMI correlated negatively with the PCr/ATP ratio.

Figure 5: Sample cardiac phosphorus spectra from (a) a young subject (with PCr/ATP = 1.95) and an older subject (with PCr/ATP = 1.55). A difference in PCr concentration is seen. The spectra are presented as acquired before correction for saturation due to heart rate, flip angle experienced at the cardiac tissue and blood content.



Positron emission tomography (PET) has been used to demonstrate that myocardial glucose uptake is inversely associated with NEFA concentration in both healthy and insulin resistant individuals (Lautamaki *et al.*, 2006). Glucose uptake is essential for glycolytic ATP production in the myocardium when the heart is stressed- low glucose uptake can lead to ischemic injury to the heart. Scheuermann-Freestone et al (2003) report that the lower cardiac PCr/ATP ratios in patients who had lower circulating plasma glucose concentrations suggested that the decreased glucose availability may have limited glucose uptake in the heart. PET–measured myocardial glucose uptake was found to be inversely correlated with liver fat (r= -0.413;p =0.001) in people with T2DM (Rijzewijk *et al.*, 2008) indicating a relationship between fatty liver and cardiac metabolism. In their study of 61 males with T2DM, patients were divided into groups depending on their level of liver fat; low <5.56%, high>5.56% (Rijzewijk *et al.*, 2008). Those with high liver fat levels were found to have significantly lower PCr/ATP and reduced myocardial glucose metabolism when compared to the group with low liver fat. However, there was no change in cardiac structure

or function in either group. The high liver fat group had a mean age of 56 ± 1 years and BMI 30.1 ± 0.6, and the low liver fat a mean age of 57 ± 1 years and BMI 27.1 ± 0.6. Thus the difference between the groups in PCr/ATP and glucose metabolism could be due to a difference in BMI. Another study in patients with T2DM found that those with higher liver fat (>8%) exhibited decreased myocardial glucose uptake and extraction (Lautamaki *et al.*, 2006). The heart may also suffer metabolic alterations in people with NAFLD, as this condition is also linked to increased blood glucose and high levels of circulating NEFA. Although disturbances in glucose control are key symptoms of both NAFLD and T2DM, it is the changes in lipid availability and oxidation which appear to impact directly on cardiac health.

The rate of myocardial lipid uptake, unlike that of glucose into myocytes, is not regulated by a hormone and therefore increasing circulating lipids increases uptake. The mechanism(s) by which fatty acids contribute to cardiac pathology are not completely understood. In patients with T2DM, obesity and NAFLD, NEFA are circulating in abundance. Fatty acid uptake by cardiac myocytes likely exceeds mitochondrial oxidative capacity and results in lipid overstorage (Carley and Severson, 2005) (i.e. cardiac steatosis or “fatty heart”). This produces lipotoxic intermediates, such as ceramide, that increase production of reactive oxygen species which, if sustained, can lead to apoptosis. BMI has been shown to correlate positively with myocardial triglyceride content in people without diabetes (Szczepaniak *et al.*, 2003). However, the relationship between BMI and myocardial triglyceride content is exaggerated in patients with impaired glucose tolerance, even before the development of T2DM (McGavock *et al.*, 2007) suggesting that lipid accumulation in the heart is an early manifestation in the pathogenesis of T2DM. These adaptations to myocardial triglyceride content were also accompanied by impaired diastolic function in both the pre-diabetes and diabetes groups. This relationship extends into T2DM, with levels of myocardial triglyceride and LV diastolic dysfunction higher than healthy controls matched for BMI and age (Rijzewijk *et al.*, 2008).

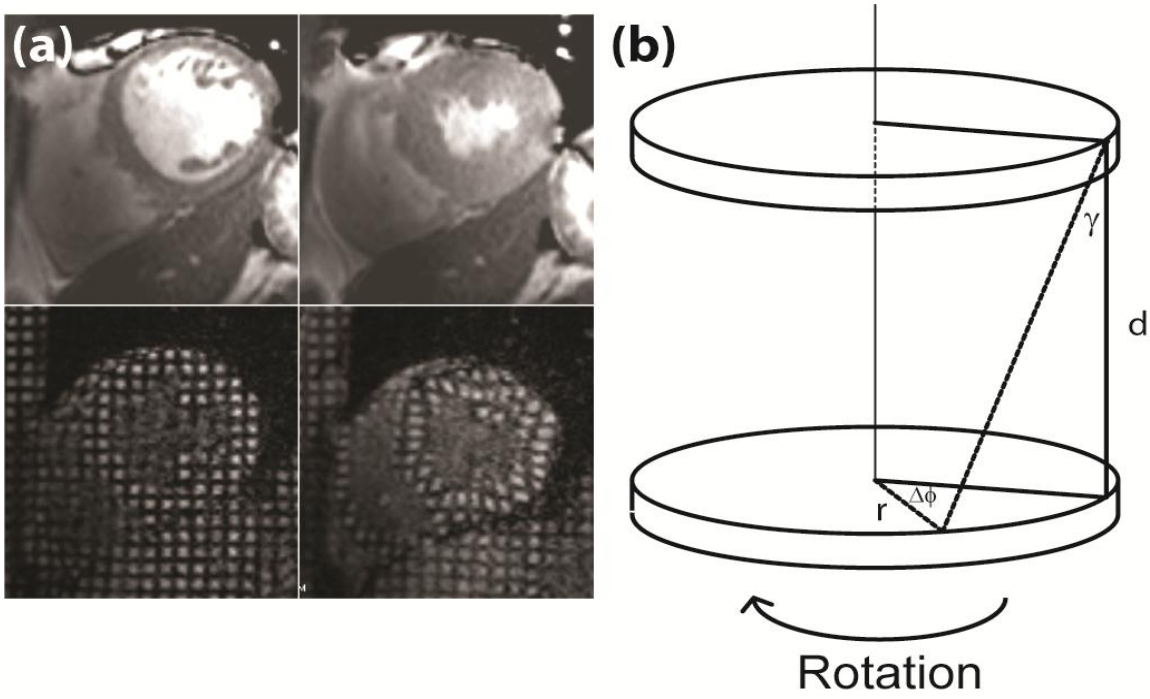
More recent imaging techniques have built upon the cardiac morphological and metabolic changes to demonstrate that the dynamic function of the heart can be used to characterise the early stresses on the heart. Magnetic resonance imaging (MRI) is considered the reference standard for non-invasive assessment of cardiac structure and provides robust measures of systolic and diastolic function in fine detail.

The E/A ratio is a first generation test for diastolic performance of the heart. The E/A ratio is the ratio between early (E) and late (atrial - A) ventricular filling velocity. In a young and compliant heart, early ventricular filling accounts for ~80% of ventricular end diastolic volume (with atrial systole pushing the last ~20% of blood into the ventricle). Thus, the 'E' component of the ratio is greater than the 'A' component. In an ageing, less compliant heart, a greater proportion of this blood is pushed into the ventricles during atrial systole. In this scenario, the emphasis of ventricular filling during late diastole increases the 'A' component of the E/A ratio causing a reversal of the ratio. The reversal of the E/A ratio is widely accepted as a clinical marker of diastolic heart failure and can be assessed using MRI techniques (Jones *et al.*, 2010).

Cardiac tagging (see Figure 6), a technique which allows the special determination of cardiac wall motion and strain in two dimensions, has allowed for the first time the measurement of the development and release of left ventricular torsion (Lumens *et al.*, 2006). Cardiac tagging enables detection of early defects in myocardial deformation by analysis of circumferential strain and torsion. In the healthy heart, torsion occurs such that there is homogeneity of fibre shortening across the myocardial wall and is a marker of the dominance of epicardial fibres over endocardial fibres as a consequence of the greater radius in the epicardium. Torsion describes the twisting motion of the heart due to opposite rotation of base and apex, and occurs as a result of equilibrium of strain across the myocardial wall, between the contraction of myofibres in the subepicardium and subendocardium. The ratio of LV torsion to endocardial

circumferential shortening (torsion-to-shortening ratio; TSR) during systole reflects the transmural distribution of contractile myofibre function. This is a sensitive marker of altered epicardial-endocardial interactions, which is constant among healthy individuals of the same age but increases with normal ageing and disease (Lumens *et al.*, 2006; Cheng *et al.*, 2009) and is thought to represent early myocardial dysfunction. With impairment of subendocardial contractile function, counteraction of torsion by contraction of subepicardial myofibres is less effective, causing net torsion to increase. Thus TSR increases with impairment of contractile function in the subendocardial layers relative to the subepicardial layers (Lumens *et al.*, 2006), suggesting there is an associated loss of local contractile myofibre function in the subendocardium relative to the subepicardium potentially caused by subclinical pathological incidents. Myocardial strains have been assessed and found to be altered in people with T2DM (Fonseca *et al.*, 2004), T1DM (Chung *et al.*, 2006) and hypertrophic cardiomyopathy (Young *et al.*, 1994) using tagging techniques, but have not yet been investigated in people with NAFLD.

Figure 6: a) Cardiac cine-imaging (*top*) and cardiac tagging (*bottom*) at diastole (*left*) and systole (*right)*, showing how a rectangular grid of nulled signal applied at diastole remains with the tissue through the cardiac cycle, allowing calculation of strain and torsion. (b) Tagging in two parallel sections allows the calculation of the torsion (the longitudinal-circumferential shear angle γ) between two short axis planes a distance d apart with radius r where one short axis plane rotates through $\Delta\phi$ relative to the other. $\gamma = \tan^{-1}[(2r \sin(\Delta\phi/2))/d]$.



1.7 Summary of Literature Review

Metabolic control is achieved via a highly responsive, finely regulated system integrating multiple organs, particularly the liver, skeletal muscle and adipose tissue, which allows the body to respond quickly to fluctuating demands and conditions. If this system becomes disrupted at any level, then metabolic disorders can develop resulting in physiological changes at muscle, liver and heart level. Levels of obesity, T2DM and NAFLD continue to rise and will place increasing pressure on health services in the future, especially since these conditions are being diagnosed in people at a younger age. Current lifestyle habits in Western countries promote metabolic disarray, with the energy balance being tipped in favour of too little energy expenditure. This balance needs redressing if metabolic disorders are to be managed effectively in the long-term, and innovative ways of empowering people to move more and sit less need to be devised.

Chapter 2: Methodology

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Chapter 2: Methodology

2.1 Recruitment Strategy

Patients with non-alcoholic fatty liver disease (NAFLD) were recruited from hepatology clinics within the Newcastle upon Tyne Hospitals NHS Foundation Trust or through advertisements in local newspapers. Healthy controls were recruited from advertisements posted throughout Newcastle University and from the Magnetic Resonance Centre database.

2.2 Informed Consent Process

Potential recruits were given the relevant patient information sheet and study synopsis prior to attending for their screening visit. This gave them time to digest the information and to formulate any questions to ask at their initial appointment. During the screening visit, details of the study were explained, and any potential risks discussed. The researcher also answered any questions regarding participation in the study at this visit. If the volunteer was happy to proceed with taking part in the study and met the inclusion criteria, they were asked to sign a consent form (see Appendix 1). At this point, the NAFLD patients were made aware that their GP would be informed about their participation in the study and also that they may withdraw at any point, without detriment to their future healthcare.

2.3 Screening Visit / Progressive Exercise Test

This visit was undertaken at the Clinical Research Facility, Royal Victoria Infirmary, Newcastle upon Tyne.

2.3.1 Physical Examination

All volunteers underwent a physical examination comprising: auscultation of the heart and lungs, evaluation of the abdomen for any abnormalities, inspection of the lower extremities for oedema and arterial pulses, an inspection of the skin (paying particular attention to the lower extremities in people with diabetes) and an assessment of reflexes.

Body weight (kg) was measured to the nearest 10g with an electronic scale (SECA 799, Birmingham, UK), and standing height (cm) measured to the nearest 0.1cm with a stadiometer (SECA 220 address as above). Both measurements were performed using standard techniques while the subject was shoeless. Body mass index (BMI) was calculated as body weight (kg)/height^2(m). Waist and hip circumference were measured to the nearest 0.5cm using a non-stretch tape measure. Waist circumference was measured at the mid-point between the lower costal margin and the level of the anterior superior iliac crests. Hip circumference was measured at the level of the greater trochanters.

Volunteers underwent a resting 12-lead electrocardiogram (ECG; Custo med GmbH, Ottobrunn, Germany) and blood pressure check (Suntech Tango+, Suntech Medical Ltd, Oxford, UK) in a seated position, to determine normal cardiac function.

Anyone found to have contraindications to exercise or exercise testing (Trenell, 2009) was excluded at this point and a letter written to their GP containing the relevant findings.

2.3.2 Progressive Exercise Test

Peak oxygen consumption was determined using an electronically braked recumbent cycle ergometer (Corival Lode BV, Groningen, The Netherlands). Following a 5 minute warm up at 25W, resistance was increased by 1W per 8 seconds until the participant could no longer maintain a cadence of 60rpm, chose to stop, or continuing was contraindicated (Trenell, 2009). The ECG was used to continuously monitor heart rhythm, and blood pressure measured every 2 minutes during the exercise test. These were monitored for at least 5 minutes after the exercise test had been terminated. Expired gases were collected using a Hans Rudolf breathing mask and analysed online for oxygen consumption, carbon dioxide elimination and ventilation (CORTEX Biophysik, Leipzig, Germany). The Metalyzer device was calibrated daily for gas volume and composition, and ambient air pressure.

This test provided measures of maximum workload (W), VO_2peak, anaerobic threshold (AT) and maximum respiratory exchange ratio (RER).

2.4 Questionnaires

2.4.1 Physical Activity Readiness Questionnaire (PARQ)

The PARQ, endorsed by the American College of Sports Medicine (ACSM), encompasses medical history, medication, current activity levels, and whether the volunteer feels there are any barriers that may prevent them from exercising. This allows the stratification of participants based on medical history/current “fitness” levels into one of three risk categories: high, moderate, and low. The PARQ allowed the team to identify volunteers for whom the intervention was inappropriate (see Appendix 2 for the full PARQ).

2.4.2 MRI Screening Questionnaire

Volunteers were asked to fill out a questionnaire to establish if magnetic resonance scanning was contraindicated, e.g. due to metal implants (see Appendix 2 for the full questionnaire).

2.4.3 International Physical Activity Questionnaire (IPAQ)

Volunteers completed the IPAQ (see Appendix 2) which asks them to recall their physical activity over the previous seven days. The IPAQ includes four activity domains: job-related physical activity, transportation, housework (including house maintenance and caring for the family), recreation and leisure time activity. It also reports time spent sitting. The questionnaire was administered when the volunteer returned their Sensewear monitor (see Section 2.5).

2.5 Physical Activity

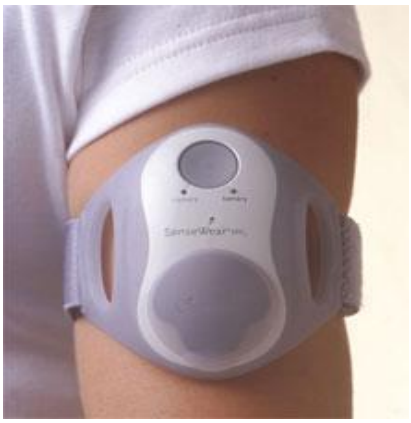
Physical activity and energy expenditure were assessed objectively using a validated (St-Onge *et al.*, 2007) multi-sensor array (SenseWear Pro_3, Bodymedia Inc, Pennsylvania, USA). Volunteers were asked to wear the arm band on their right upper arm (at the mid-humerus point of the triceps) for seven days. The armband produced the following data as units per day: total energy expenditure; active energy expenditure; average metabolic equivalents (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.

The portable armband uses a biaxial accelerometer, a heat flux sensor, a galvanic skin response sensor, and a near-body ambient temperature sensor to

capture data. These data as well as the volunteer's date of birth, height, body weight, sex, hand dominance and smoking status (smoker or non-smoker) were used to calculate energy expenditure .

All subjects were instructed to remove the armband only for bathing/showering purposes or any water activity.

Figure 7: Physical activity and energy expenditure were assessed objectively using a validated multi-sensor array



2.6 Whole Body Composition

Whole body composition was determined using air displacement plethysmography using a BodPod (Life Measurement Inc., Concord, CA, USA). This was calibrated before each measurement using a known calibration standard. The technique has been validated against the reference standard of hydrostatic weighing, dual-energy X-ray absorptiometry and bioelectrical impedance in healthy and overweight/obese adults (Sardinha *et al.*, 1998; Biaggi *et al.*, 1999; Fields *et al.*, 2005). All patients had their body composition measured whilst fasted and were asked to wear tight fitting underwear and don a lycra cap (provided by the manufacturer) to minimise the effects of hydration/recent food intake and air trapping respectively. The manufacturers indicate that the general error range of the Bodpod is 1-2% (the same as hydrostatic weighing).

Figure 8: Body composition of patients was measured using a BodPod

2.7 Magnetic Resonance Imaging and Spectroscopy

Imaging was undertaken at the Magnetic Resonance Centre at Newcastle University's Campus for Ageing and Vitality, Newcastle upon Tyne. Magnetic resonance studies were performed using a 3.0 Tesla Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands) - see Figure 9.

2.7.1 Liver Fat Measurement

Following an 8 hour fast, intrahepatic lipid (IHL) was measured by localised proton magnetic resonance spectroscopy (^{1}H-MRS) placing one "large" voxel (compared to a biopsy) in the inferior right lobe of the liver, well away from the liver surface and large intrahepatic vessels (to avoid weighting the spectrum artificially by "water") (PRESS, TR/TR = 3000 ms/35 ms, 3 x 3 x 3cm voxel, SENSE torso array, 6 signal averages). Using one large voxel, as opposed to 2-3 smaller ones, improves the signal to noise ratio and minimises patient time in the magnet (Szczepaniak *et al.*, 2005). Blinded quantification of the spectra (water and CH_2 resonances) was performed using the java-based magnetic

resonance user interface (jMRUI version 3.0) (Naressi *et al.*, 2001a; Naressi *et al.*, 2001b). Following manual phase correction, spectra were analysed using a non-linear least squares algorithm (AMARES) (Vanhamme *et al.*, 1999). IHL was expressed as a percentage of liver volume, corrected for proton density of water and lipid (Longo *et al.*, 1995) using the following equation:

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

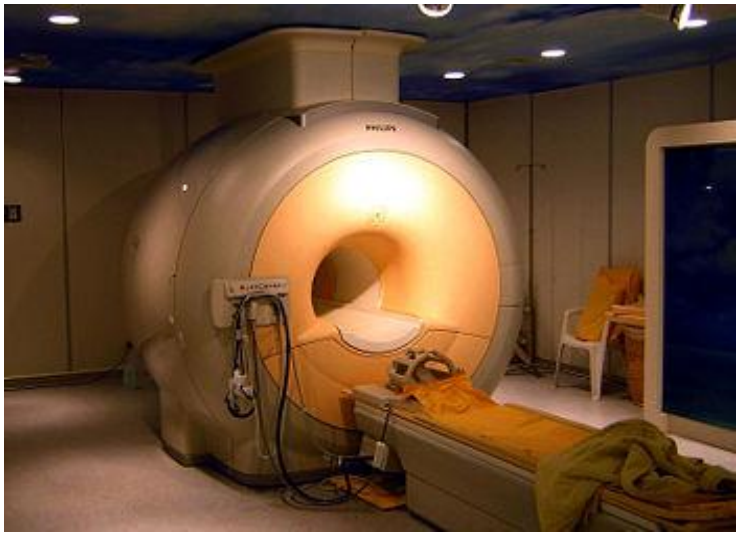
where V_f and V_w are the volumes of the fat and water phases, respectively, and D_f and D_w are the proton-density values of the fat and water phases, respectively. A value for D_f of 110mol/L was used and for D_w 111mol/L. FTSA is the ratio of the detectable fat signal peak area to the total signal peak area and N_v is the ratio between the signal integrals in the two spectral regions containing lipids and was found to be 0.85 (Longo *et al.*, 1995).

2.7.2 Abdominal Fat Measurement

Subcutaneous and visceral fat content was performed by acquiring images at the L4/L5 junction using a 3-point Dixon sequence (TR/TE/number of averages/flip angle = 50ms/3.45, 4.60, 5.75ms/1/30º, matrix 160x109, median FOV 440mm, range 400-480mm to suit subject size with 70% phase FOV). The slice was acquired during a breath-hold and with slice thickness 10mm (Donnelly *et al.*, 2003; Shen *et al.*, 2004). Fat and water were separated, and binary gating applied to produce a map of structures containing more than 50% fat, identified as the subcutaneous and visceral fat. A watershed algorithm was used to divide the binary image into distinct areas and allowed easy separation of the subcutaneous and visceral fat. ImageJ (Abramoff *et al.*, 2004) was used to subtract the two areas to produce the area of visceral fat.

All images were analysed by the same investigator who was blinded as to the participants' identification.

Figure 9: Magnetic resonance studies were performed using a 3.0 Tesla scanner



2.8 Glucose Control

Following an 8 hour overnight fast a cannula was inserted into a forearm vein. A 75g glucose load (Lucozade Original; GlaxoSmithKline, Brentford, UK) was consumed within five minutes. Blood samples were taken at time 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90 and 120 minutes. Samples were analysed immediately for whole blood glucose (YSI 2300 Stat Plus-D; Yellow Springs Instruments, Yellow Springs, Ohio, USA). Blood samples for NEFA and insulin were spun in a centrifuge (Harrier 18/80R; MSE Ltd, London, UK) for 10 minutes at 3000rpm at 4ºC. Plasma was then pipetted off each sample and stored in a freezer (Sanyo Biomedical freezer; Loughborough, UK) at -40ºC. Samples were batch analysed (to increase intra-rater reliability and decrease inter-rater variability) when all samples had been collected. Plasma NEFA (NEFA-HA; Wako Ltd, Osaka, Japan), and insulin (Coat-A-Count Insulin RIA kit, Diagnostic Products Corporation, California, USA) were analysed in Newcastle University's accredited laboratory.

Area under the curve (AUC) for the resulting glucose response profile was calculated using the trapezoidal rule (Le Floch *et al.*, 1990) and insulin resistance determined using the homeostasis model assessment of IR (HOMA-IR) which is a mathematical model for determining IR from fasting glucose and insulin concentrations which has been validated by the euglycemic-hyperinsulinemic clamp (Bloomgarden, 2006):

HOMA-IR= ((fasting glucose x fasting insulin)mmol/L)/22.5)

NEFA suppression (NEFA-S) was assessed during the fsOGTT and the 0-30min change used as a measure of NEFA-S (Patel *et al.*, 2005).

Fasting samples were also analysed in a Clinical Pathology Accredited laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry) for: liver enzymes (ALT, AST, GGT), lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol), triglycerides and HbA1c. Serum samples were collected in silica clot activator polymer gel containing vacutainers (BD Diagnostics, Plymouth, England) - total cholesterol, triglycerides, and liver enzymes were measured using a Roche Modular P and test kits (Roche Diagnostics Ltd, Burgess Hill, UK). HbA_{1c} was measured using a TOSOH HLC-723G7 (Tosoh Corporation, Tokyo, Japan).

2.9 Resting and Exercise Stimulated Lipid Oxidation

Following an overnight fast (≥ 8 hours with no food or beverages), a cannula was inserted into a forearm vein for blood draws. Resting substrate oxidation was determined by expired gas analysis (CORTEX Biophysik , Leipzig, Germany) using a Hans Rudolf breathing mask while participants lay supine for 30 minutes in a quiet room, without speaking or sleeping and with minimal movements. The first 15 minutes were an acclimatization period and the second 15 minutes were used to determine resting substrate oxidation. The calorimeter gas analysers were calibrated before every measurement for gas

volume and composition, and ambient air pressure. A resting blood sample was taken and analysed for glucose, NEFA and insulin levels.

Exercise stimulated lipid oxidation was measured in the fasted state during a 60 minute cycle. Participants were asked to perform a 5 minute warm up on the recumbent cycle ergometer at 25W. The resistance on the ergometer was then increased to 50% of the maximum workload achieved during their maximal exercise test (performed at the screening visit). The participant then cycled at this resistance for 60 minutes (60-70rpm). Expired air was collected every 15 minutes. Respiratory quotient (RQ) was calculated from VO_2 / VCO_2.

Venous blood was collected every 15 minutes during exercise. After the 60 minutes, the participant completed a 5 minute cool down at 25W. A further blood sample was taken one hour after the exercise had been completed (the participant remained fasted during this time).

Substrate oxidation rates and energy expenditure were calculated from oxygen consumption and carbon dioxide production values using stoichiometric equations (Frayn, 1983). Calculation of substrate oxidation during normal circumstances:

$$VO_2 \text{ (l/min)} = 0.746c + 2.03f + 6.04n$$

$$VCO_2 \text{ (l/min)} = 0.746c + 1.43f + 4.89n$$

where c is grams of carbohydrate (as glucose) oxidised per minute, f grams of fat per minute and n is grams of urinary nitrogen excreted per minute. Nitrogen can be estimated as excretion of 1mol of urea (28g N) is equivalent to synthesis of 1mol of glucose and use of 1mol CO_2.

Whole blood glucose was measured immediately after sampling (as detailed previously in Section 2.8); all other blood samples (NEFA and insulin) were

centrifuged, plasma removed then frozen and stored to await batch-analysis. Insulin and NEFA were processed as previously described.

Chapter 3: Measuring energy expenditure in adults with non-alcoholic fatty liver disease: evaluation of a portable device

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Chapter 3: Measuring energy expenditure in adults with non-alcoholic fatty liver disease: evaluation of a portable device

3.1 Introduction

Obesity and lifestyle-related disease levels are rising dramatically, and the assessment of energy expenditure (EE) as a tool in the regulation of body weight is of critical importance. In 2008, almost 25% of adults in England were classified as obese (NHS, 2010), and in 2004 in the US, obesity affected up to one-third of adults (Ogden *et al.*, 2006). Lifestyle interventions focusing on weight loss remain the cornerstone of non-alcoholic fatty liver disease (NAFLD) management (Day, 2006; Harrison and Day, 2007). However, weight loss is difficult to achieve and sustain by dietary means (Tamura *et al.*, 2005; Bellentani *et al.*, 2008; Oza *et al.*, 2009) and other therapeutic avenues are necessary. It must be considered that physical activity could potentially assist in reducing liver fat independent of weight loss. Cross-sectional studies report that low levels of physical activity are associated with higher levels of liver fat (Perseghin *et al.*, 2007a; Zelber-Sagi *et al.*, 2008; St. George *et al.*, 2009). However, understanding of the role of physical activity and EE in moderating liver fat, and hence its use as a therapeutic intervention, is limited by the ability to accurately measure physical activity and EE. To date, there are no validated systems for the assessment of physical activity and EE specifically for NAFLD.

Self-reported physical activity is valid (Craig *et al.*, 2003; Hagströmer *et al.*, 2006; Bull *et al.*, 2009) but its imprecision limits it to understanding broad differences in physical activity in cross-sectional studies. These techniques are limited in their ability to observe changes in activity patterns or allow accurate determination of EE. The gold standard method for the assessment of EE in free-living individuals is doubly labelled water (DLW).

The DLW method determines EE from the proportional breakdown of ingested stable isotope labelled oxygen and hydrogen. This method measures carbon

dioxide production by comparing the rates of turnover of oxygen and hydrogen of the ingested water. The underlying principle is that hydrogen, ingested in water, is lost from the body only in the water form as urine and as water vapour released during exhalation, but that the oxygen is lost both in the same way and also in expired carbon dioxide. Therefore, the difference in the elimination rate can be used to determine carbon dioxide production, which in turn can be related to EE. The ultimate goal of the DLW method is to provide estimates for total EE. Although accurate in determining EE, this method does not provide information about patterns of activity and also requires serial urine sample collection, limiting the ability to deploy the technique in large numbers of people. Indirect-calorimetry methods can accurately assess EE in laboratory conditions, although these methods cannot be used to assess free-living energy expenditure because of the technical restrictions of wearing the gas analysers (Bluck, 2008).

As a result of the limitations of using the reference techniques, portable activity monitors are being increasingly used to objectively monitor physical activity levels and EE in healthy people and chronic disease populations. Pedometers are simple devices, which use up and down motions as estimates of steps, but fail to capture intensity, frequency or duration of activity or those activities that do not involve stepping. Basic, uniaxial accelerometers measure acceleration of the body or body parts in one plane and take into account the speed, direction and duration of movements and convert these to movement counts to allow for estimation of EE. Biaxial or triaxial accelerometers provide information about movement in multiple planes, allowing an improved estimate of physical activity EE than uniaxial units (Plasqui and Westerterp, 2007). Numerous multi-sensor arrays have recently been developed. These devices may hold benefits over accelerometry alone by determining EE from a mixture of movement via biaxial accelerometer, temperature and galvanic skin responses which are more sensitive to changes in movement efficiency and combining these with data about the wearer, including age, height and weight (Liden *et al.*, 2002). A multi-sensor array consisting of a biaxial accelerometer, skin temperature, air temperature and galvanic skin response sensors, has been validated against

DLW for use in the general adult population (St-Onge *et al.*, 2007), but not in people with NAFLD. EE may be different in people with NAFLD, as people tend to be overweight or obese, and generally carry out less activity than their healthy counterparts (Zelber-Sagi *et al.*, 2008).

The primary aim of this study was to evaluate a portable multi-sensor array for measuring daily and physical activity EE compared with DLW in free-living adults with NAFLD.

3.2 Subjects and Methods

Daily total energy expenditure (TEE) was measured in 10 subjects with NAFLD over a 10-day period simultaneously with a portable multi-sensor array and DLW. Subjects were recruited from hepatology clinics within the Newcastle upon Tyne Hospitals NHS Foundation Trust or through advertisements in local newspapers. NAFLD was defined as >5% liver fat on ^{1}H-MRS. Baseline characteristics of the group can be found in Table 2. Exclusion criteria included: heart or kidney disease; implanted ferrous metal; insulin sensitising treatment or dietary change (for people with Type 2 diabetes, diet and metformin were acceptable for inclusion if stable for six months); and alcohol intake above 21 units for men or 14 units for women. The study protocol was approved by Newcastle & North Tyneside 1 Research Ethics Committee. All subjects provided written informed consent.

Body weight (kg) and height (cm) were measured as described in Section 2.3.1. The subject then provided a urine sample to act as the pre-dose sample which allowed us to determine the background level of isotope enrichment in the environment.

Doses for the DLW were calculated relative to body weight from weighted aliquots of $^{2}H_2O$ and $H_2{}^{18}O$. The dose given was 70mg/kg of $^{2}H_2O$ and 174mg/kg of $H_2{}^{18}O$. This dosing regimen allowed for accurate measurement of the isotopes at the end of the sampling period, when as little as 10% of the isotope remains in the body water. Using this dosing regimen, total EE can be measured with a coefficient of variation of <5% (Bluck, 2008). The portable multi-sensor array (SenseWear Pro_3, Bodymedia Inc, Pennsylvania, USA) was fitted to the subject's right upper arm after DLW administration.

The subject was required to collect a urine sample at approximately the same time each morning for the next 10 days (preferably the second void of the day)

and refrigerate these at home. Subjects were asked to wear the portable multi-sensor array continuously over this 10-day period (Plasqui and Westerterp, 2007) and were instructed to remove the monitor only for bathing/showering purposes or any water-based activity. A subject's Sensewear data was deemed acceptable for analysis if overall wear time was ≥85% of the total time that they had the monitor *in situ* (Mackey *et al.*, 2011).

DLW analysis was carried out using isotope ratio mass spectrometry as described previously (Hoffman *et al.*, 2000) at the MRC Human Nutrition Centre in Cambridge, UK .

Physical activity energy expenditure (PAEE) was calculated using the formula:

$$PAEE = 0.9 \times TEE - BMR$$

which removes the energy expenditure due to the thermic effect of meals (assumed to be 10% of TEE) and energy expenditure devoted to basal metabolic rate (BMR). BMR was not assessed directly by the multi-sensor array so BMR was calculated using Harris-Benedict equations (Harris and Benedict, 1919):

$$BMR\ (men) = 66.5 + (5.0 \times H) + (13.8 \times W) - (6.8 \times A)$$

$$BMR\ (women) = 655.1 + (1.9 \times H) + (9.6 \times W) - (4.7 \times A)$$

where H is height in cm, W is weight in kg, and A is age in years.

3.2.1 Statistical Analysis

All analyses were performed using SPSS version 19 (SPSS Inc, Chicago,US). Bland-Altman analyses were used (Bland and Altman, 1986) to examine differences between DLW and the portable multi-sensor array. Specifically, individual comparisons between DLW and the portable multi-sensor array were completed by examining a plot of differences in total EE by the DLW and the armband versus mean total EE determined by both methods. From these data, limits of agreement between DLW and the armband were calculated, defined as the mean difference between the two methods ± 2SD of the difference. Paired t-

tests were performed to determine differences between mean values for total EE and physical activity EE for the portable multi-sensor array and DLW. To examine the strength of association between the portable multi-sensor array and DLW estimates, Pearson correlation coefficients were calculated. Regression analyses were conducted between daily total EE measured by the portable multi-sensor array and DLW, and physical activity EE measured by the two techniques. Statistical significance was set at $p<0.05$. Data are presented as mean ± SD.

3.3 Results

Baseline characteristics are presented in Table 2. Data are presented from nine patients as one of the portable multi-sensor arrays failed to record any data during the monitoring period. Average wear time for the monitor was good (95 ± 5%).

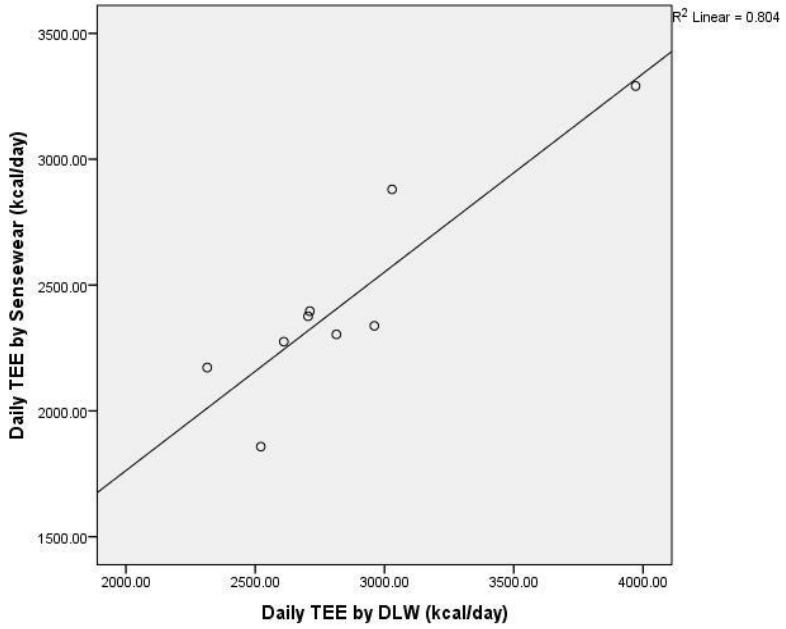
Table 2: Baseline characteristics; values are given as means (SD)

	n=9
Age (years)	56 (15)
Weight (kg)	91 (10)
Height (cm)	165 (8)
BMI (kg/m²)	33 (5)
Liver fat, %	14 (6)
Daily average TEE from DLW (kcal)	2849 (474)
Daily average TEE from Sensewear (kcal)	2432 (417)
Mean difference daily average TEE (DLW - Sensewear; kcal)	416 (210)

There was a positive correlation between DLW and the multi-sensor array when measuring daily total EE ($r = 0.896$; $p < 0.01$ - see Figure 10). Regression analysis showed a high level of agreement between the multi-sensor array and DLW measurements of daily total EE ($r^2=0.80$; $p<0.01$).

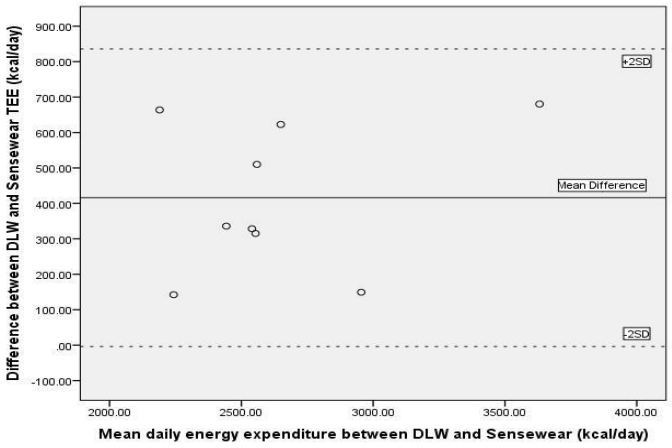
The multi-sensor array recorded significantly lower daily physical activity EE than the DLW method (530 ± 225 vs. 920 ± 284: $p<0.01$).

Figure 10: Relationship between DLW and the multi-sensor array methods for measuring daily total EE (n=9; p<0.01)



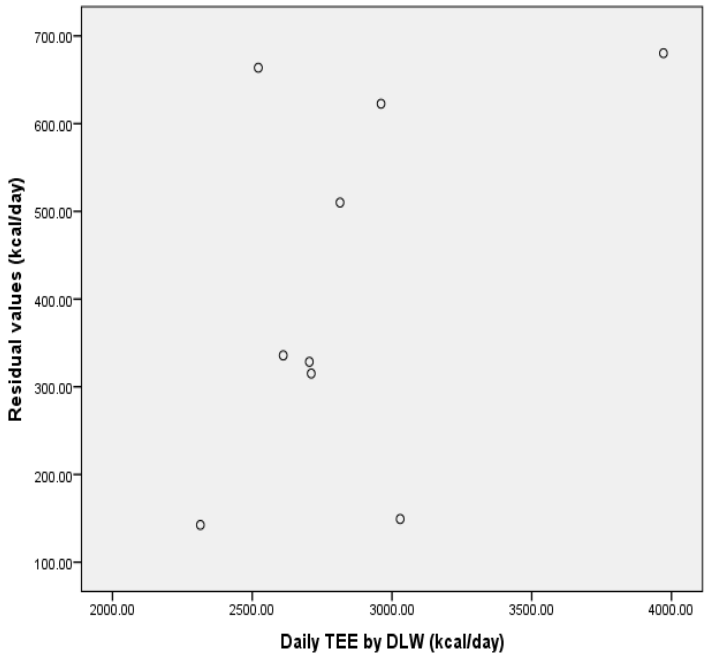
The Bland-Altman plot for total EE is shown in Figure 11. In adults with NAFLD, the portable multi-sensor array consistently underestimated daily total EE by an average 416kcal/day when compared with DLW (2432 ± 417 vs. 2849 ± 474kcal/day; p<0.01). Using our pre-defined limits of agreement (mean ± 2SD), all subjects fell within these limits. However, this represents a large range in estimates of total EE (-4 to 836kcal/day) which would be clinically unacceptable. When taking into account potential analytic and biological variation, within subject measures of DLW can vary by approximately 200kcal/day and when comparing two methods, it has been suggested that an additional 100kcal/day be added to create a 300kcal/day limit of agreement (St-Onge *et al.*, 2007). Using this limit of agreement between the 2 methods, only 2 out of 9 subjects (22%) fell under this cut-off within our study.

Figure 11: Bland-Altman plot between DLW and the multi-sensor array methods for measuring daily energy expenditure (n=9). The broken horizontal lines represent the limits of agreement corresponding to mean ± 2SD.



There was no significant correlation between total EE and the size of error between the two measures (see Figure 12). This suggests that the multi-sensor array underestimated calorie turn over at all activity levels seen within this cohort.

Figure 12: Difference in daily total energy expenditure (EE) between the two methods plotted against the reference method for measuring daily total EE (n=9; p>0.05)



3.4 Discussion

This is the first study to evaluate a portable multi-sensor array simultaneously against DLW in obese adults with clinically defined NAFLD. These data demonstrate that the multi-sensor array systematically underestimated energy expenditure in this patient group. However, the monitor may still be a useful tool for promoting physical activity as part of a lifestyle intervention in the clinical setting.

Despite the important role of EE in metabolic control and weight maintenance, little is known about the role of EE in NAFLD. Physical activity levels in NAFLD have, to date, only been measured using self-report questionnaires (Perseghin *et al.*, 2007a; Zelber-Sagi *et al.*, 2008; St. George *et al.*, 2009). These studies report that low levels of physical activity are associated with higher levels of liver fat. However, although questionnaires provide a useful description of what people are doing, studies comparing estimates of EE from validated questionnaires against DLW describe a systematic underestimation of EE (Gardner and Poehlman, 1998; Conway *et al.*, 2002; Maddison *et al.*, 2007; Ishikawa-Takata *et al.*, 2011). These differences have been attributed to physical activity questionnaires not including key activities related to active EE (such as climbing stairs, personal care and sedentary activities), differences in data sampling between questionnaires and DLW, and inaccurate assignment of metabolic equivalents to self-reported activities (Neilson *et al.*, 2008). These subjective methods are also subject to reporting error, linked to recall and social desirability bias, and are inaccurate in determining frequency, duration and intensity of physical activity (Warren *et al.*, 2010). As such, studies focussing on subjective reports should be taken with caution and more accurate means of determining EE are needed.

The portable multi-sensor array offers an accessible objective means of determining both levels of physical activity and EE. However, the present study demonstrates that the portable multi-sensor array detected a mean 416kcal/day

(14.4%) underestimation of total EE when compared with DLW in this group of patients with clinically identified NAFLD. A previous study using the same multi-sensor array and DLW reported a mean 117kcal/day underestimation by the multi-sensor array in adults with normal BMI (24 ± 4kg/m^2) (St-Onge *et al.*, 2007). This study also appeared to show a progressive underestimation of EE as BMI increased. In line with this, the multi-sensor array has been reported to underestimate resting EE by 8.8% when compared with indirect-calorimetry in obese adults (BMI 42 ±7) (Papazoglou *et al.*, 2006). Combined, these data suggest that these portable multi-sensor arrays are prone to underestimation of TEE and resting EE and that this inaccuracy is augmented in people that are obese. This underestimation in obese adults may be due to the changes in body temperature and galvanic skin response with increased fat mass which, in turn, affect the accuracy of the data being fed into the algorithms within the sensor. Irrespective of the reasons why the algorithms underestimate EE, these data suggest that caution should be given to using these sensors in obese people to determine EE.

Physical activity EE is the most variable component of total EE (Warren *et al.*, 2010). Daily physical activity EE was underestimated by 390kcal/day (42.4%) by the portable multi-sensor array when compared to DLW. As the portable multi-sensor array does not provide a direct estimate of physical activity EE, this was determined using the same methods used in the DLW studies (Harris-Benedict estimates of BMR). The underestimation of both BMR and total EE combine to produce a magnified inaccuracy in estimating physical activity EE over and above the inaccuracies in total EE. Other studies using Sensewear monitors against DLW have also found underestimations of physical activity EE in the general adult population (St-Onge et al 2007:218kcal/day) and in older adults (Mackey et al 2011: 156kcal/day using Sensewear 6.1; 108kcal/day using Sensewear 5.1). Combined with the inaccuracies in estimating total EE, the specific and magnified underestimation of physical activity EE further reduces the applicability of the devices in determining EE in overweight people with NAFLD.

The portable multi-sensor array was easy to use by the subjects, provided minimal discomfort, and very little interference with daily activity. However, these monitors are not waterproof, so needed to be removed for bathing/showering and any water-based activity. This means that the calorie turn over for these activities is not captured by the multi-sensor array, although the monitors do estimate EE for off-body time using pre-programmed proprietary algorithms developed by the manufacturer that take into account data from the sensor and characteristics of the wearer.

A clear limitation of this study is the small sample size. This was restricted as the study was of an exploratory nature and studies with larger patient numbers are needed to ascertain the accuracy of the multi-sensor array definitively in people with NAFLD. Generally, the use of DLW is dictated by the prohibitive monetary costs of using the technique. In an obese patient group, larger quantities of the $^{2}H_{2}O$ and $H_{2}{}^{18}O$ were needed to dose the heavier people. Urine samples were analysed for each day of the 10-day period to determine daily loss of each isotope. This increases the accuracy of the results rather than relying on before- and 10 day-after-dose samples (Bluck, 2008), but obviously resulted in increased costs of sample analysis. The decision to “scale-down” this validation study in terms of sample size, makes it difficult to generalise results to the wider population but does provide preliminary data on how accurate the monitors are in measuring EE in people with NAFLD.

In conclusion, the Sensewear portable multi-sensor array was an easy-to-use method of objectively measuring activity levels in free-living adults. However, the results show that the portable multi-sensor array significantly underestimated measures of total and active energy expenditure in obese people with NAFLD. If the portable multi-sensor arrays are to be used in obese subjects, it is necessary to incorporate new, obesity-specific algorithms in the device’s software. The portable devices may prove useful tools in engaging obese people with NAFLD in physical activity within lifestyle interventions and aid in positive behaviour change. However, the interpretation of results in obese

individuals needs to be undertaken with caution as the devices are prone to significant underestimation in total EE and physical activity EE. Thus, the multi-sensor arrays may be a good motivational tool to use with patients within the clinical setting, but may not be an accurate research tool.

Chapter 4: Physical activity levels in adults with non-alcoholic fatty liver disease

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Chapter 4: Physical activity levels in adults with non-alcoholic fatty liver disease

4.1 Introduction

Physical activity is a key determinant of metabolic control and is commonly recommended for people with non-alcoholic fatty liver disease (NAFLD), usually alongside weight loss and dietary change (Harrison and Day, 2007). Even though physical activity and exercise are recommended as part of treatment for NAFLD, there have been no large-scale studies with adequate statistical power to guide health practitioners in prescribing exercise programmes or for generating physical activity guidelines for the management of these patients. Evidence for the benefit of physical activity comes from prospective studies showing that individuals who maintain a physically active lifestyle are less likely to develop insulin resistance (IR), impaired glucose tolerance, or T2DM (Boule *et al.*, 2001; Snowling and Hopkins, 2006; Thomas *et al.*, 2006; Colberg *et al.*, 2010). Cross-sectional studies also suggest that people with NAFLD have lower levels of physical activity that those without (Hsieh *et al.*, 1998; Perseghin *et al.*, 2007a; Zelber-Sagi *et al.*, 2008).

Self-reported physical activity levels have been shown to be lower in people with NAFLD than their “healthy” counterparts (Hsieh *et al.*, 1998; Perseghin *et al.*, 2007a; Zelber-Sagi *et al.*, 2008) and links have been made between low cardiorespiratory fitness and NAFLD severity (Church *et al.*, 2006; Krasnoff *et al.*, 2008). However, these subjective methods in determining physical activity are also subject to reporting error, linked to recall and social desirability bias, and are inaccurate in determining frequency, duration and intensity of physical activity (Warren *et al.*, 2010).

Increasing physical inactivity is becoming a growing problem in the general population (Blair, 2009) and low levels of physical activity are compounded by

an increase in physical inactivity. Physical inactivity, including activities such as sitting, is reported to be higher in people predisposed to the metabolic syndrome, excessive adiposity and T2DM (Dunstan *et al.*, 2004; Dunstan *et al.*, 2005; Levine *et al.*, 2005; Healy *et al.*, 2008). Consequently, increases in sedentary time could play a potential role in the development of or predisposition towards NAFLD independent of physical activity / exercise and needs to be considered when introducing lifestyle interventions.

To date, no studies have reported the relationship between *objectively* measured physical activity levels, IHL and metabolic control in people with NAFLD. This study determined the level of objectively measured physical activity and sedentary time, in people with NAFLD and investigated links between physical activity, IHL, glucose control and body composition.

4.2 Subjects and Methods

Thirty-three sedentary (≤60 minutes of vigorous activity per week) adults with clinically defined NAFLD were recruited to the study from hepatology clinics within the Newcastle upon Tyne Hospitals NHS Foundation Trust or through advertisements in local newspapers. NAFLD was defined as >5% IHL on ^{1}H-MRS (see Section 2.7.1). General descriptions can be found in Table 3. Exclusion criteria included: heart or kidney disease; implanted ferrous metal; insulin sensitising treatment or dietary change (for people with T2DM, diet and metformin were acceptable for inclusion if stable for six months); and alcohol intake above 21 units for men or 14 units for women. An age- and sex-matched healthy control group were recruited through advertisements at the University.

The study protocol was approved by County Durham and Tees Valley 2 Research Ethics Committee. All participants provided written informed consent. Visits were undertaken at the Clinical Research Facility, Royal Victoria Infirmary, or the Magnetic Resonance Centre, both in Newcastle upon Tyne, UK.

Physical activity: Physical activity and energy expenditure were assessed objectively using a multi-sensor array (SenseWear Pro$_3$, Bodymedia Inc, PA, USA – see Section 2.5 for further details) previously validated in healthy adults (St-Onge *et al.*, 2007). Volunteers were asked to wear the armband on their right upper arm (at the mid-humerus point of the triceps) for seven days. All subjects were instructed to remove the armband only for bathing/showering purposes or any water-based activity. A subject's multi-sensor array data were acceptable for analysis if overall wear-time was ≥95% of the total time that they had the monitor in situ (St-Onge *et al.*, 2007)

The following matrices of physical activity were derived from the multi-sensor array as units per day: total energy expenditure (TEE); active energy expenditure (AEE); average metabolic equivalents (METs – see Section 1.5 for

definition); 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 monitor worn.

Volunteers completed the validated (Hagströmer *et al.*, 2006) International Physical Activity Questionnaire (IPAQ; see Appendix 2) to determine levels of physical activity and sitting time. The IPAQ includes four activity domains: job-related physical activity, transportation, housework (including house maintenance and caring for the family), recreation and leisure time activity. The questionnaire was administered when the volunteer returned their multi-sensor array so activity recorded on both should cover the same time period. The IPAQ was scored using the guidelines produced by The IPAQ Group (www.ipaq.ki.se/scoring.pdf).

Anthropometry: Body weight (kg) and standing height (cm) were measured as described in Section 2.3.1. In the NAFLD group, body composition was measured using air displacement plethysmography (BodPod, Life Measurement Inc., CA, USA) – see Section 2.6 for further details (Sardinha *et al.*, 1998; Biaggi *et al.*, 1999; Fields *et al.*, 2005).

Glucose Control and Liver Enzymes: In the NAFLD group, a blood sample was taken from a forearm vein following a >8 hour overnight fast. Whole blood glucose was measured immediately (YSI 2300 Stat Plus-D, Yellow Springs Instruments, Yellow Springs, OH). HbA_{1c} was measured using a TOSOH HLC-723G7 (Tosoh Corporation, Tokyo, Japan) and ALT using a Roche Modular P and test kits (Roche Diagnostics Ltd, Burgess Hill, UK) in a Clinical Pathology Accredited laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry).

4.2.1 Statistical Analysis

Statistical analysis was performed using SPSS version 19 (SPSS Inc, Chicago, US). Between group differences were evaluated using a paired t-test and Pearson's correlation was used to investigate associations between variables. Statistical significance was set at $p<0.01$ to allow for multiple comparisons. Data are mean ± SD unless otherwise stated.

4.3 Results

Seven data sets were excluded as the volunteers wore the monitor for less than 95% of the 7-day period. Therefore, 26 data sets were analysed in each group.

The groups were well matched for age (54 ± 13 vs. 54 ± 13years; p=0.088) and sex (p=1.000). Weight and BMI were significantly higher in the NAFLD group when compared with controls - general descriptions can be found in Table 3.

Table 3: Subject characteristics (activity data from multi-sensor array); values are given as means (SD)

	NAFLD (n=26)	Control (n=26)	p-value
Age (years)	54 (13)	54 (13)	0.088
Weight (kg)	93 (12)	86 (15)	**0.007**
Height (cm)	171 (9)	175 (10)	0.021
Body Mass Index (kg/m^2)	32 (5)	28 (5)	**0.001**
Intrahepatic lipid (%)	13.7 (7.5)	-	-
ALT (U.L^{-1})	53 (33)	-	-
Fasting Glucose	5.7 (1.8)	-	-
HbA1c	6.2 (0.9)	-	-
Body Fat (%)	38.6 (9.0)	-	-

(ALT, alanine aminotransferase)

Average daily MET levels were significantly lower in the NAFLD group when compared to controls (1.2 ± 0.2 vs. 1.4 ± 0.2 METs; p<0.01) as was active energy expenditure (classed as activity of >3.0 METs: 453 ± 293 vs. 713 ± 315kcal; p<0.01). People with NAFLD spent less time performing physical activity of any intensity (76 ± 51 vs. 121 ± 48mins/day; p<0.01) than the controls, and a significant difference was also observed between the groups when the physical activity was divided up into intensity levels – see Table 4 for more details. Sedentary time, classed as activities up to 3.0 METs, was

significantly higher in the NAFLD group (1337 ± 51 vs. 1294 ± 55mins/day; p<0.01).

Table 4: Physical activity data recorded by multi-sensor array (data reported as daily means (SD))

	Control (n=26)	NAFLD (n=26)	p-value
Duration on body (min)	1413 (17)	1412 (15)	0.812
Lying (min)	480 (96)	495 (60)	0.522
Sleep (min)	374 (81)	398 (63)	0.211
TEE (kcal)	2853 (485)	2692 (453)	0.060
Steps	9987 (3513)	8281 (3243)	0.061
Average METs	1.4 (0.2)	1.2 (0.2)	**0.001**
Sedentary time (min)	1294 (55)	1337 (51)	**0.004**
AEE (kcal)	713 (315)	453 (293)	**0.001**
Physical activity duration (min)	121 (48)	76 (51)	**0.001**
Moderate activity (min)	109 (41)	74 (49)	**0.003**
Vigorous activity (min)	6 (8)	2 (4)	**0.010**
Very vigorous activity (min)	4 (9)	0 (0)	0.050

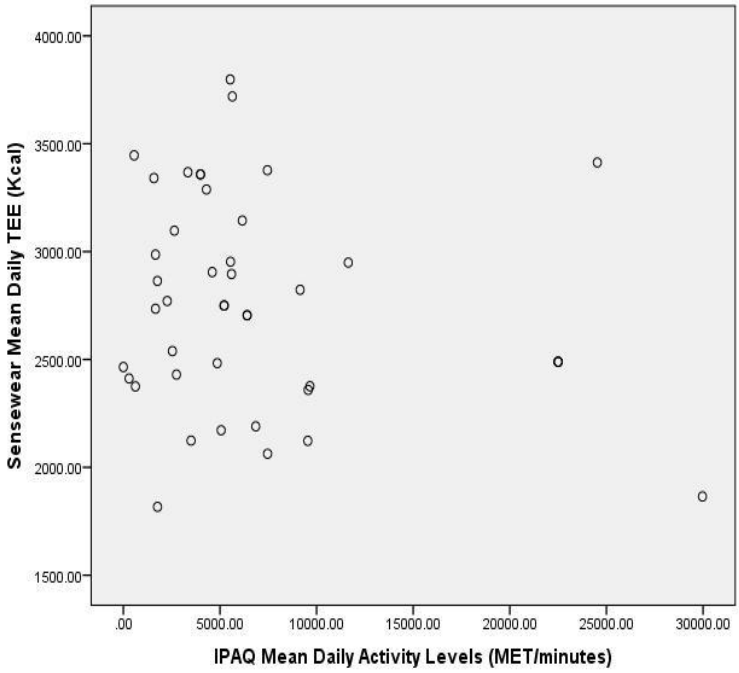
(TEE, total energy expenditure; METs, metabolic equivalents; AEE, active energy expenditure)

Using the self-reported IPAQ, people with NAFLD reported lower levels of physical activity and more time spent sitting than their healthy counterparts (see Table 5). There was no correlation between the daily TEE recorded by the multi-sensor array and physical activity levels reported in the IPAQ across the whole group (r= -0.192; p=0.216 – see Figure 13). Sedentary time measured by the multi-sensor array was not associated with sitting time reported in the IPAQ (r= 0.278; p=0.071).

Table 5: Physical activity data reported using the IPAQ

	Control (n=18)	NAFLD (n=18)	p-value
Mean daily MET-minutes	8783 (8968)	5806 (5635)	0.267
Mean daily sitting time (mins)	277 (107)	364 (182)	0.131

Figure 13: Association between self-reported physical activity using the IPAQ, and objectively measured physical activity using the multi-sensor array



BMI was negatively correlated with average METs (r= -0.535; p<0.01) and physical activity duration (r= -0.494; p< 0.01) in NAFLD. BMI was positively associated with sedentary time but this did not reach statistical significance (r= 0.435; p=0.026). Body fat percentage showed negative correlations with TEE (r= -0.549; p<0.01), steps (r= -0.536; p< 0.01), average METs (r= -0.699; p<0.01), AEE (r= -0.609; p<0.01) and physical activity duration (r= -0.611; p<0.01). There was a positive association between body fat percentage and sedentary time (r= 0.536; p<0.01). There was no correlation between IHL, fasting glucose, HbA1c and ALT with any of the physical activity parameters measured by the multi-sensor array within the NAFLD group.

4.4 Discussion

This is the first study to objectively measure physical activity levels and sedentary time in adults with clinically defined NAFLD, and to use this data to investigate the relationship between physical activity, IHL and metabolic control. The data reveals that people with NAFLD spend more time physically inactive and achieved lower levels of physical activity than people without NAFLD. Levels of physical inactivity or physical activity were not associated with the severity of liver fat or glucose control in this small well-characterised group.

Sedentary behaviour or physical inactivity is a growing health problem, silently putting people at heightened risk from a host of chronic diseases (WHO, 2003; Blair, 2009). Adults with NAFLD spend more time pursuing sedentary behaviours than those without fatty liver and these patterns of inactivity can be clearly observed using the activity traces produced by the Sensewear monitors (see Figures 14a and 14b). This increase in physical inactivity may compound the detrimental health effects caused by lack of physical activity. In the present study, adults with NAFLD accumulated 22.5 hours per day of sedentary activity. Sedentary behaviours involving sitting or lying down are characterised by a low MET value of less than 3, and are related adversely to metabolic biomarkers and to poorer health outcomes (Sugiyama *et al.*, 2008). Sitting for prolonged periods reduces the opportunity for cumulative energy expenditure produced by muscle contractions as we move around throughout the day (Hamilton *et al.*, 2007), and impairs the exercise/muscle contraction stimulated uptake of glucose from the circulation and lipoprotein lipase activity thus hampering fat handling. High levels of overall physical inactivity may contribute to obesity and metabolic disorders, potentially as much as lack of moderate-vigorous physical activity. Even if adults meet the public health guideline for leisure-time physical activity, they may have a high risk of becoming overweight or developing metabolic disorders if they spend a large amount of time in sedentary behaviours during the rest of the day (Levine *et al.*, 2005; Sugiyama *et al.*, 2008). Combined, these results demonstrate for the first time that physical inactivity is prominent in NAFLD. Targeting these periods of inactivity may constitute an effective means of improving liver lipid.

Figure 14a: Activity trace (derived from Sensewear) for a 63 year old female without NAFLD (Red lines demonstrate EE; green lines represent steps)

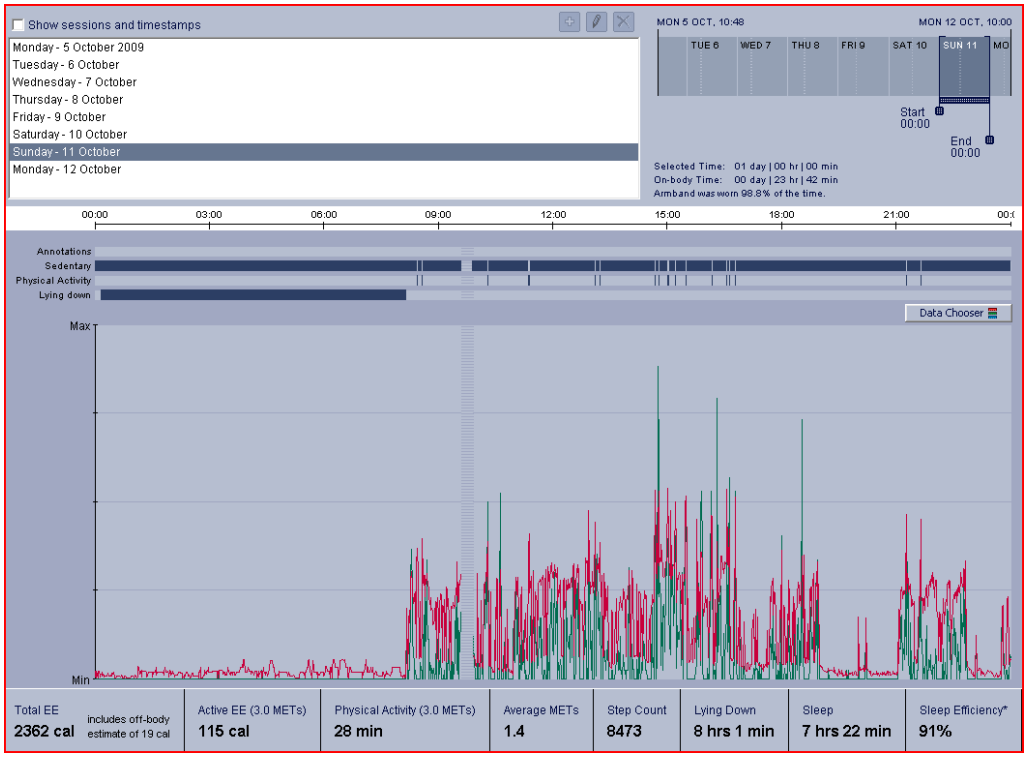
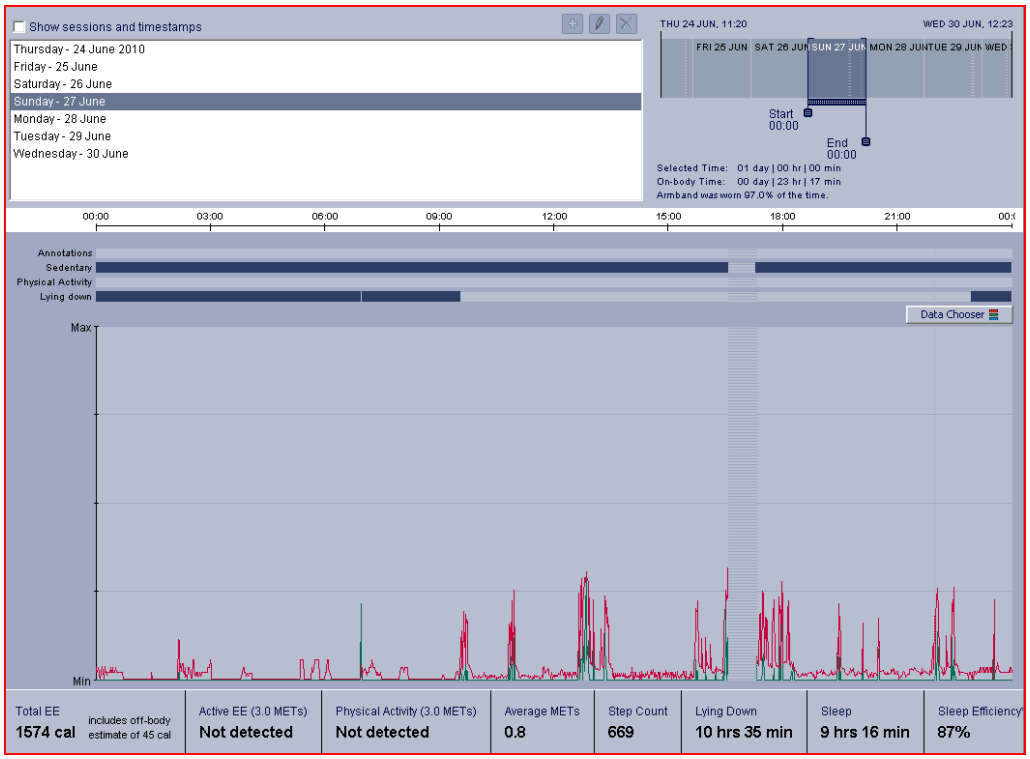


Figure 14b: Activity trace (derived from Sensewear) for a 63 year old female with NAFLD



The present data also highlights that people with NAFLD undertake less daily physical activity than their healthy counterparts. This is demonstrated by lower levels of total calories expended, lower levels of active EE and less steps taken compared with the healthy controls. Physical activity levels in NAFLD have to date only been measured using self-report questionnaires (Perseghin *et al.*, 2007a; Zelber-Sagi *et al.*, 2008; St. George *et al.*, 2009). These observations consistently report that low levels of self-reported physical activity are associated with higher levels of liver lipid. However, these subjective methods have significant limitations and are subject to recall and social desirability bias and are inaccurate in determining frequency, duration and intensity of physical activity (Warren *et al.*, 2010). The poor associations between objective and subjective reports of physical activity (IPAQ) in the present study highlight the importance of objectively assessing physical activity. The link between physical activity and liver lipid highlights the positive effects of a physically active lifestyle upon IR, impaired glucose tolerance and T2DM (Eriksson and Lindgärde, 1991; Helmrich *et al.*, 1991). Physical activity should, theoretically, aid the prevention and/or progression of NAFLD through its reciprocal relationship with glucose control. Despite the disparity between objective and subjective reports of physical activity, the same message remains, low levels of physical activity are associated with higher levels of liver lipid.

People with NAFLD not only carry out a lower average level of physical activity, but also undertake less moderate and vigorous activity than people without NAFLD. The lower levels of these higher intensity activities may have implications as the intensity of the activity may also play a key role in improving metabolic control. However, the reports demonstrating that higher intensity activities / exercises are linked to improvements in metabolic control are not unequivocal. One meta-analysis found exercise intensity was not associated with a difference in HbA1c in people with T2DM (Boule *et al.*, 2001). However, when using resistance training independently, moderate-high intensities were associated with greater improvements in muscle bulk and overall glucose control (Gordon *et al.*, 2009) and high-intensity interval training was shown to improve hyperglycemia in patients with T2DM (Gillen *et al.*, 2012). Harrison and

Day (2007) speculated that moderate exercise, performed 3-4 times per week, expending about 400kcal each time seemed adequate to augment improvement in the metabolic profiles of patients with NAFLD. However, although useful clinical guidelines, the evidence underlying these suggestions is lacking. We might hypothesise that by increasing EE throughout the day, and decreasing sedentary time, if patients can accumulate an extra 400kcal of non-exercise activity thermogenesis (NEAT) they may achieve similar metabolic benefits. Examples of calorific expenditure for a person of 68kg performing the activities for one hour are light gardening 330Kcal; walking at 3.5mph 280Kcal; general housework 270Kcal; shopping for groceries 180Kcal (www.nutribase.com/exercala.htm). This may be a useful guide when prescribing physical activity within the clinic setting. There is no clear evidence on which exercise approach is best in improving metabolic control. However, the pragmatic approach is likely the successful one. Reducing sedentary behaviour, increasing NEAT and increasing moderate intensity exercise are all good for metabolic control. The one which is most successful is the one which the patient is most likely to achieve.

In patients with NAFLD, BMI and body fat percentage were negatively correlated with objectively measured markers of increasing physical activity and positively associated with sedentary time. In obesity, studies have shown similar findings (Williamson *et al.*, 1993; Di Pietro, 1999; Jiménez-Pavón *et al.*, 2010), whereby the more overweight/obese people are, the less physical activity they undertake, which drives the vicious cycle of increasing weight gain. Currently, there is no evidence to suggest whether the initial weight gain drives the physical inactivity or vice versa.

A major limitation of using the Sensewear multi-sensor array was that it consistently underestimated energy expenditure in people with NAFLD when compared with DLW (see Chapter 3). The energy expenditure data is used to generate the results for all the other activity parameters, apart from steps, and thus the results must be received with caution. The error margin is such that it

could reduce the overall differences observed within the groups. Using the multi-sensor array clinically with an individual looking to change their activity habits as part of a lifestyle intervention, should provide comparable data for the same person at different time-points, thus showing the effects of behaviour change on a 1:1 basis. These results should not diminish the importance and quality of the physical inactivity data.

The mechanisms by which a physically active lifestyle may moderate liver fat relate predominantly to metabolic regulation. Changes in circulatory metabolites and hormones, such as glucose, lipids and insulin have a direct impact upon IHL. Insulin and physical activity/exercise are the two most physiologically important stimulators of skeletal muscle glucose transport. Both increase skeletal muscle glucose uptake by encouraging translocation of GLUT4 to the muscle cell wall (Hayashi *et al.*, 1997; Röckl *et al.*, 2008). GLUT4 expression and recruitment to the plasma membrane increase in response to exercise training, thus facilitating glucose uptake into the trained muscle (Hayashi *et al.*, 1997). Reduced muscle perfusion and mass as a result of physical inactivity reduces peripheral glucose uptake and may precede IR and T2DM (Wang *et al.*, 2009). However, these changes in muscle can be reversed with a general increase in activity levels enhancing glucose storage. Enhanced glucose uptake and storage, through muscle contraction and increased storage capacity, reduces the levels of circulating insulin required to maintain glucose homeostasis. A decrease in insulin levels reduces *de novo* lipogenesis in the liver, thus reducing hepatic steatosis (Tamura *et al.*, 2005; Lavoie and Gauthier, 2006).

Physical activity has also been shown to improve IR through positive changes in fat oxidation in muscle, which cannot be achieved by energy restriction alone (St. George *et al.*, 2009). Mitochondria are the main organelles involved in fat oxidation within the cells, and their content and functional capacity has been shown to be improved by lifestyle interventions (Toledo *et al.*, 2007). Mild-moderate intensity exercise (25-65% of VO_2max) is associated with a 5-10 fold

increase in fat oxidation above resting amounts because of increased energy requirements of muscle, enhanced fatty acid availability and increased mitochondrial capacity (Horowitz and Klein, 2000). This intensity of exercise coincides with numerous day-to-day activities, thus it may be reasonable to suppose that increasing daily NEAT may hold the same physiological benefits for increasing fat oxidation. An increase in whole-body fat oxidation will decrease circulatory NEFA and thus reduce the delivery of fatty acids to the liver via the portal vein, thus decreasing fat storage within the liver.

Although our results from the DLW validation study show a propensity for the multi-sensor array to significantly underestimate daily energy expenditure in NAFLD, these monitors are still a useful tool to glean insights into free-living daily activity patterns in these people (see Figure 14). The movement counts provided by the multi-sensor array provide an alternative measure for physical activity than kcals, and the activity-traces allow us to informally look at periods of sedentary behaviour and breaks within this. The MET levels provided also act as a guide as to the intensity of activity undertaken which allows clinicians to tailor advice to this. Volunteers found the monitors easy to use and unobtrusive, with little impact on daily activity. Limitations of these monitors are that they are not waterproof and thus need to be removed for any water-based activity, and occasionally people developed mild skin irritations to the straps after wearing them for a prolonged time.

The use of physical activity monitors in the clinical environment may provide clinicians with a way to engage patients in discussion about activity/exercise. Data recorded can be used as a baseline measure from which to tailor subsequent physical activity counselling and build appropriate exercise programmes. Their use offers the opportunity to provide immediate feedback to patients when they return to clinic, by providing a short report or a more in-depth daily analysis of activity, from which discussions about lifestyle change and weight loss can materialise. Since the visual data being presented by the clinician represents the patient’s actual day-to-day life, this may act as a

valuable tool to aid in improving adherence, patient motivation and improve clinical outcomes.

In conclusion, people with NAFLD spent more time physically inactive and less time physically active on a daily basis than people without fatty liver. The use of portable multi-sensor arrays within the clinical setting may improve physical activity participation. Often patients are not aware how much physical activity they actually engage in, so an objective measure will provide this feedback, and thus allow personal activity goals to be established in order to achieve their individual health targets. Low levels of physical activity represent a therapeutic target which may improve metabolism and prevent a progression of metabolic conditions in people with NAFLD.

Chapter 5: Cardiac structure, function and energetics in adults with non-alcoholic fatty liver disease

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Chapter 5: Cardiac structure, function and energetics in adults with non-alcoholic fatty liver disease

5.1 Introduction

The clinical impact of non-alcoholic fatty liver disease (NAFLD) clearly extends beyond the liver. People with NAFLD are exposed to double the risk of cardiovascular (CV) disease compared to people without NAFLD (Ekstedt *et al.*, 2006). Even people with raised alanine aminotransferase (ALT), without a clear clinical diagnosis of NAFLD, are exposed to an increase in the 10-year risk of CV disease (Schindhelm *et al.*, 2007) with age- and sex-adjusted hazard ratios for CV events and coronary heart disease of 1.40 (1.09-1.81) and 2.04 (1.35-3.10) respectively in the upper tertile of ALT. Furthermore, an 18 year prospective study of 132 patients with biopsy-proven NAFLD, demonstrated that CV deaths were the second most common cause of death in NAFLD patients, with rates equalling those of liver-related deaths and trailing only cancer-related deaths (Matteoni *et al.*, 1999). As such, cardiac health is of as high importance to people with liver disease and their care teams as liver health itself.

Despite the importance of cardiac health in NAFLD, to date, there has been very little research into the cardiac status of people with NAFLD. An early study of cardiac health in people with NAFLD reported alterations in cardiac morphology and energetics in young men with newly-diagnosed NAFLD using magnetic resonance techniques (Perseghin *et al.*, 2008). Cardiac morphology was not different in those with NAFLD compared to those without fatty liver, however, cardiac metabolism (assessed using ^{31}P-magnetic resonance spectroscopy) was significantly different in the NAFLD group, demonstrated by a decrease in PCr/ATP ratio. The authors suggested that in NAFLD, abnormalities in cardiac metabolism may precede the development of functional and structural re-modelling of the heart. However, it is difficult to generalise the results of this study to the usual NAFLD population as this only included young (mean age 35), normotensive, non-obese (mean BMI 27.5) males and excluded people who had other endocrine/metabolic disease. Given the central role of the

liver in metabolic control, it is not surprising that the key mediating effects of the liver upon cardiac health appears to be through its influence in metabolic control.

Magnetic resonance imaging (MRI) is considered the reference standard for non-invasive assessment of cardiac structure and provides robust measures of systolic and diastolic function in fine detail. Cardiac tagging allows the special determination of movement and stresses in two dimensions, and has permitted the torsional power generation of the heart to be examined for the first time (Lumens *et al.*, 2006). ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) permits evaluation of myocardial bioenergetics, and hence metabolic efficiency, by calculation of the PCr/ATP ratio (Crilley *et al.*, 2003). These techniques used in combination, provide us with a unique way of non-invasively investigating the intricacies of cardiac structure and function in people with NAFLD, and of potentially highlighting therapeutic targets on which to base future interventions.

The purpose of this study was to investigate whether people with non-advanced clinically defined NAFLD demonstrate alterations in cardiac structure, function and energetics using cardiac cine imaging, tagging and ^{31}P-MRS compared with healthy controls.

5.2 Subjects and Methods

Twenty three adults (12 male; 11 female) with NAFLD were recruited from hepatology clinics within the Newcastle upon Tyne Hospitals NHS Foundation Trust or through advertisements in local newspapers. NAFLD was defined as >5% IHL on ^{1}H-MRS (see Section 2.7.1). General descriptions can be found in Table 6. Exclusion criteria included: heart or kidney disease; implanted ferrous metal; insulin sensitising treatment or dietary change (for people with T2DM, diet and metformin were acceptable for inclusion if stable for six months); and alcohol intake above 21 units for men or 14 units for women. Subjects were individually age- and gender- matched with controls without clinically identified metabolic disease. The study protocol was approved by Newcastle & North Tyneside 1 Research Ethics Committee. All participants provided written informed consent.

All subjects had no previous history of cardiac disease and were screened with a 12-lead ECG (Custo med GmbH, Ottobrunn, Germany) and resting blood pressure measurements (Suntech Tango+, Suntech Medical Ltd, Oxford). Bodyweight and height were measured using an electronic scale and stadiometer respectively (SECA, Birmingham, UK). Subjects underwent an MR protocol of MR cine imaging, cardiac tagging and phosphorus cardiac spectroscopy, performed at a single session. All evaluations were completed at the Clinical Research Facility, Royal Victoria Infirmary, Newcastle upon Tyne or at the Newcastle Magnetic Resonance Centre, Newcastle upon Tyne.

5.2.1 Cardiac Magnetic Resonance Imaging

Cardiac examinations were performed using a 3T Philips Intera Achieva scanner (Best, Netherlands). A dedicated 6-channel cardiac coil (Philips, Best, Netherlands) was used with the subjects in a supine position and ECG gating. Short axis balanced steady-state free precession images were acquired covering the left ventricle (FOV = 350mm,TR/TE = 3.7/1.9ms, acceleration

factor 17, flip angle 40^{o}, slice thickness 8mm, 0mm gap, 14 slices, 25 phases, resolution 1.37mm).

Image analysis was performed using the cardiac analysis package of the ViewForum workstation (Philips, Best, Netherlands). Manual tracing of the epicardial and endocardial borders was performed on the short axis slices at end-systole and end-diastole (Figure 6a). The basal slice was selected for end-diastole and for end-systole for the left ventricle when at least 50% of the blood volume was surrounded by myocardium. The apical slice was defined as the last slice showing inter-cavity blood pool. Papillary muscles were included in the mass and excluded from the volume calculations. The inter-ventricular septum was included as part of the left ventricle (Hudsmith *et al.*, 2005). Details of the algorithm for contour selection and calculating left ventricular mass, systolic and diastolic parameters have been previously published (Jones *et al.*, 2010). The eccentricity ratio of the LV mass to the end-diastolic volume was calculated as this parameter is a measure of concentric remodelling.

5.2.2 Cardiac Spectroscopy

Cardiac high-energy phosphate metabolism was assessed using ^{31}P-MRS. Data were collected using the same 3T Intera Achieva scanner with a 10cm diameter ^{31}P surface coil (Pulseteq, UK) for transmission/reception of signal. Subjects were placed in a prone position and moved into the magnet so their heart was at magnet isocentre. Localising images were collected using the in-built body coil to confirm location of the heart. Shimming was performed using a cardiac triggered, breath-held field map (Schar *et al.*, 2004). A slice-selective, cardiac gated 1-dimensional chemical shift imaging (1D-CSI) sequence was used with a 7cm slice selective pulse applied foot head to eliminate contamination from the liver, with spatial pre-saturation of lateral skeletal muscle to avoid spectral contamination. 16 coronal phase-encoding steps were used, yielding spectra from 10mm slices (TR = heart rate, 192 averages at the centre of k-space with cosine-squared acquisition weighting, approx. 20 mins acquisition time). Spectral locations were overlaid onto an anatomical image and the first spectrum arising entirely beyond the chest wall was selected. Quantification of PCr, the γ resonance of ATP and 2, 3-diphosphoglycerate

(DPG) was performed using the AMARES time domain fit routine in the jMRUI processing software. After fitting, the ATP peak area was corrected for blood contamination by 1/6 of the amplitude of the combined 2,3-DPG peak (Conway *et al.*, 1998), and the PCr/ATP ratios were calculated and corrected for saturation, with T_1 values of cardiac PCr and ATP taken from the literature (Tyler *et al.*, 2008). Flip angle correction was made using a gadolinium-doped 20mM phenyl phosphonic acid phantom at the centre of the coil and a calibration dataset (Haase *et al.*, 1984; Buchli and Boesiger, 1993).

5.2.3 Cardiac Tagging

Tagged short axis images were acquired. Cardiac tagging works by applying radiofrequency pulses to cancel MR signal from the myocardium in diastole in a rectangular grid pattern and tracking the deformation of these tags through the rest of the cardiac cycle (Figure 6b). A turbo-field echo sequence with acceleration factor 9 was used (TR/TE/FA/NEX = 4.9/3.1/10^{o}/1, SENSE factor 2, FOV 350x350mm, voxel size 1.37x 1.37mm, tag spacing of 7mm). Two adjacent short-axis slices of 10mm thickness were acquired at mid-ventricle with a 2mm gap. 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 epi-cardial contours. Circumferential strain and the rotation of the two planes were calculated throughout the cardiac cycle. Circumferential strain is quoted for both the whole myocardial wall and the endocardial third of the wall thickness. The epicardial torsion between the two planes (taken as the circumferential-longitudinal shear angle defined on the epicardial surface) was calculated (Buchalter *et al.*, 1990). 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 maximum torsion gradient in diastole (deg/ms, defined as the maximum slope of the torsion curve in diastole), the torsion recoil rate (which is normalised for peak torsion, %/ms), and the residual torsion at 150% of the end-systolic time.

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 myocardial wall thickness at systole and diastole were determined at the same level as the cardiac tagging, and radial thickening was calculated.

5.2.4 Statistical Analysis

Between group differences were evaluated using unpaired t-tests and Pearson's correlations used to investigate associations between variables. Statistical testing was performed using SPSS version 19 (SPSS Inc, Chicago, US). Statistical significance was assumed at $p<0.01$ to allow for multiple comparisons. Data are presented as mean ± SD. Based on changes reported from a previous study in NAFLD (Perseghin *et al.*, 2008), the study was powered at 90% to detect at least a 10% difference between groups in PCr/ATP ratio, ejection fraction, E/A ratio and end-diastolic wall mass.

5.3 Results

The groups were well matched for age (54 ± 15 vs. 54 ± 15years; p=0.921) and sex (p=1.000). Physical and metabolic characteristics can be found in Table 6. There was a significant difference in systolic (130 ± 12 vs. 146 ± 16mmHg; p<0.01) and diastolic (80 ± 9 vs. 90 ± 12mmHg; p<0.01) blood pressure between the groups however, there was no correlation between blood pressure and other cardiac parameters, in particular LV mass and LV index. There was no significant difference in weight, BMI or body surface area between the groups (BSA - the parameter used for indexing throughout the study).

Table 6: Subject characteristics; values are given as means (SD)

Parameter	Controls (n=23)	NAFLD (n=23)	p-value
Age (years)	54 (15)	54(15)	0.921
SBP (mmHg)	130 (12)	146 (16)	**0.001**
DBP (mmHg)	80 (9)	90 (12)	**0.002**
Height (cm)	169 (11)	168 (9)	0.690
Weight (kg)	76 (11)	84 (14)	0.030
Body mass index ($kg \cdot m^{-2}$)	27 (4)	30 (4)	0.037
Body surface area (m^2)	1.8 (0.2)	1.9 (0.2)	0.052
Glucose ($mmol.L^{-1}$)	5.1 (0.4)	5.0 (0.8)	0.739
Triglycerides ($mmol.L^{-1}$)	1.6 (0.9)	1.7 (1.0)	0.923
Total cholesterol ($mmol.L^{-1}$)	5.4 (0.8)	5.2 (1.3)	0.692
ALT ($U.L^{-1}$)	23 (12)	51 (36)	**0.004**
Intrahepatic lipid (%)	2.4 (1.1)	10.1 (4.9)	**0.001**

Cardiac structure and function (see Table 7)

People with NAFLD had a significantly lower stroke volume index (32 ± 5 vs. 39 ± 10ml/m²; p<0.01) than healthy controls. There were no significant differences in ejection fraction, stroke volume, resting heart rate, and cardiac output between the two groups.

People with NAFLD had significantly thicker LV walls at systole (14 ± 3 vs. 12 ± 2mm; p<0.01) and diastole (8 ± 1 vs. 7 ± 1mm; p<0.01) than those without fatty liver and showed decreased longitudinal shortening (14 ± 3 vs. 17 ± 3%; p<0.01). The eccentricity ratio was significantly higher in the NAFLD group (1.2 ± 0.2 vs. 0.9 ± 0.2g/ml; p<0.01) indicating concentric remodelling. LV mass (sum of wall mass and papillary muscles) was similar in both groups. Wall mass (excluding papillary muscles) was no different between the groups, however, the NAFLD group had a significantly higher mass of papillary muscles (11 ± 6 vs. 6 ± 3g; p<0.01). Within the patient group, no significant linear correlations were shown between systolic or diastolic blood pressure and any markers of LV mass and wall thickness. Longitudinal shortening was negatively associated with wall thickness at systole (r= -0.520; p<0.01), the eccentricity ratio (r= -0.603; p<0.01) and peak circumferential strain (r= -0.571; p<0.01) in the NAFLD group but not in the control.

Neither end-diastolic volume (EDV), nor end-systolic volume (ESV) were significantly different between the groups. When these values were indexed to take into account the individuals' BSA, both EDV index and ESV index were significantly lower in the NAFLD group (p<0.01 for both parameters – see Table 6).

Table 7: Cardiac structure and function; values are given as means (SD)

Parameter	Controls (n=23)	NAFLD (n=23)	p-value
Ejection fraction (%)	60 (6)	64 (7)	0.060
Heart rate (bpm)	58 (9)	63 (10)	0.102
Stroke volume (ml)	71 (19)	62 (13)	0.060
Stroke volume index (ml/m²)	39 (10)	32 (5)	**0.005**
Cardiac output (l/min)	4.1 (0.8)	3.8 (0.7)	0.339
End diastolic volume (ml)	120 (35)	100 (31)	0.045
End systolic volume (ml)	49 (18)	38 (20)	0.056
Wall mass (g)	96 (27)	102 (28)	0.445
Papillary muscle (g)	7 (3)	11 (6)	**0.004**
LV mass (g)	103 (28)	113 (32)	0.248
LV mass indexed (g/m²)	56 (13)	58 (12)	0.575
EDV indexed (ml/m²)	65 (18)	51 (14)	**0.004**
ESV indexed (ml/m²)	26 (9)	19 (9)	**0.011**
Wall thickness systole (mm)	12 (2)	14 (3)	**0.001**
Wall thickness diastole (mm)	7 (1)	8 (1)	**0.002**
Radial wall thickening (%)	63 (20)	72 (32)	0.260
Longitudinal shortening (%)	17 (3)	14 (3)	**0.005**
Eccentricity ratio (g/ml)	0.9 (0.2)	1.2 (0.2)	**0.001**
Early filling percentage (%)	70 (11)	63 (14)	0.057
E/A ratio	2.0 (1.4)	1.5 (2.4)	0.203
Early diastolic filling rate (ml/s)	312 (111)	260 (90)	0.083
Late diastolic filling rate (ml/s)	192 (73)	221 (82)	0.211

Diastolic function, represented by the E/A ratio and early filling percentage, was not statistically significant between the groups however did exhibit a tendency to be lower in NAFLD. The lower the E/A ratio, the higher the torsion-to-shortening ratio (TSR) (r= -0.406; p=0.05) and peak torsion (r= -0.418; p=0.05) in NAFLD and controls (TSR: r= -0.468; p=0.04; peak torsion: r= -0.451; p=0.05). Reduced longitudinal shortening was associated with reduced early filling percentage (r= 0.462; p=0.03) in NAFLD but not in controls. Markers of diastolic function did not correlate to PCr/ATP ratio in either group, nor did baseline measures of BMI, fasting glucose, triglycerides and total cholesterol.

Cardiac torsion and strain (see Table 8)

Peak whole wall strain was higher in the NAFLD group (20 ± 3 vs. 17 ± 3%; p<0.01), as was peak endocardial strain (28 ± 4 vs. 23 ± 5%; p<0.01) when compared with controls. TSR was not significantly different between the groups. Circumferential systolic rate was higher in the NAFLD group when compared to controls (0.10 ± 0.02 vs. 0.08 ± 0.02; p<0.01) but there was no difference no difference in the rate that torsion was released.

Cardiac ^{31}P-MR spectroscopy

PCr/ATP ratio was not significantly reduced in NAFLD when compared with controls (1.75 ± 0.31 vs. 1.89 ± 0.28; p=0.133).

Correlations between modalities

There were no correlations between liver fat %, triglyceride levels or glucose with cardiac structure or function in the NAFLD group.

Table 8: Cardiac torsion and strain; values are given as means (SD)

Parameter	Controls (n=23)	NAFLD (n=23)	p-value
Peak endocardial circumferential strain (%)	23 (5)	28 (4)	**0.001**
Peak whole wall circumferential strain (%)	17 (3)	20 (3)	**0.013**
Torsion to shortening ratio (rad)	0.53 (0.20)	0.43 (0.20)	0.052
Peak torsion (deg)	6.6 (1.8)	7.0 (2.1)	0.547
Rate of systolic torsion	0.03 (0.01)	0.02 (0.01)	0.476
Torsion recoil rate (%/ms)	0.4 (0.1)	0.4 (0.2)	0.581
Rate of torsion release	0.02 (0.01)	0.01 (0.01)	0.045
Circumferential systolic rate	0.08 (0.02)	0.10 (0.02)	**0.001**
Circumferential diastolic rate	0.05 (0.01)	0.05 (0.02)	0.458

Secondary analysis

In order to examine whether the differences shown were due to NAFLD per se and not due to associated obesity, the groups were retrospectively matched for BMI by removing outliers (n=20 NAFLD, n= 23 control: BMI 29 ± 3 vs. 27 ± 4 $kg \cdot m^{-2}$; p=0.170). Between group differences in cardiac structure and function were the same as prior to group manipulation. Matching the groups for BP was not possible as 20/23 people with NAFLD had above the mean systolic BP for the controls, and 11/23 had above the diastolic average.

5.4 Discussion

This is the first study to examine cardiac status in a clinical group of NAFLD patients using the combined techniques at 3.0T of phosphorous spectroscopy, cardiac tagging and cine MRI to measure cardiac energetics, torsion and circumferential strain, and morphology. The major findings in NAFLD patients compared to age- and gender-matched controls are: 1) there was no significant difference in cardiac energetics; 2) there was thickening of the cardiac wall, independent of changes in LV mass; 3) altered myocardial strains occur without a change in torsion or TSR; 4) concentric remodeling is apparent; and 5) there was evidence of early diastolic dysfunction. All of these changes were observed in the absence of overt cardiac disease.

This study demonstrated alterations in morphology before there was evidence of any changes in cardiac metabolism. There was no significant difference in PCr/ATP ratio between the NAFLD and control groups, although the NAFLD group showed a decrease of 7% compared to controls. This may be due to the heterogeneous nature of our NAFLD and control groups in terms of age and BMI. A previous study in young men with newly-diagnosed NAFLD (Perseghin *et al.*, 2008), found a significant decrease of 12% in the NAFLD group compared with controls (1.84 ± 0.34 vs. 2.11 ± 0.32; p= 0.016). It has been postulated that changes in cardiac metabolism occur as a result of high levels of circulating NEFA (Scheuermann-Freestone *et al.*, 2003; Lautamaki *et al.*, 2006; Larsen and Aasum, 2008) and that these fatty acids are preferentially utilised by the heart as a fuel decreasing the PCr/ATP ratio. We did not see a significant difference between the groups in the PCr/ATP ratio within the present study, however, given that there were no variations in the levels of triglycerides or total cholesterol (surrogate biomarkers for NEFA) between the NAFLD and control groups the lack of difference in metabolic efficiency may not be surprising. Interestingly, both groups had relatively high baseline levels of both markers which may have resulted in an overall lowering of PCr/ATP in both groups when compared to the other study in NAFLD (Perseghin *et al.*, 2008). The lack of correlation between liver fat, PCr/ATP ratio and other cardiac parameters

suggests there may not be a linear association between liver and cardiac metabolism *in vivo* in humans and that other non-liver mediators are present.

Structurally, the present results demonstrate a general thickening of the LV wall in NAFLD, independent of changes in LV mass. Increased wall thickness was associated with reduced longitudinal shortening which is consistent with findings in other clinical groups (Young *et al.*, 1994; Fonseca *et al.*, 2004; Hollingsworth *et al.*, 2011). Total wall mass was greater in the NAFLD group, although not significantly, but a larger proportion of this was represented by papillary muscle. This thickening of the wall may be due to the increased strains seen in NAFLD, both affecting the endocardium and entire wall. The small, but significant, absolute increase in papillary muscle mass in the NAFLD group is likely some statistical aberration as opposed to any physiological phenomenon. In NAFLD, the reduction in end- systolic and end-diastolic blood pool volumes, alongside an elevated eccentricity ratio is indicative of concentric remodelling with greater wall thickness and normal chamber size. These findings are supported in obese subjects without known fatty liver (Peterson *et al.*, 2004; Wong *et al.*, 2004).

Studies investigating cardiac morphology in obesity have found increased LV mass and wall thickness, both positively associated with an increase in BMI (Lauer *et al.*, 1991; Peterson *et al.*, 2004; Wong *et al.*, 2004; Powell *et al.*, 2006; Turkbey *et al.*, 2010). The present data reports moderate positive correlations between BMI and wall thickness at systole ($r= 0.299$; $p<0.05$) and radial wall thickening percentage ($r= 0.346$; $p<0.05$) but not LV mass. The extent of cardiac remodelling in obesity increases with its severity and duration, and is exacerbated by concomitant hypertension and the plasma volume expansion seen with increasing body size (Vasan, 2003; Abel *et al.*, 2008). Diastolic dysfunction was also a common finding (Iacobellis *et al.*, 2002; Peterson *et al.*, 2004; Wong *et al.*, 2004; Powell *et al.*, 2006) in obesity, however systolic function seemed to be preserved (Vasan, 2003; Wong *et al.*, 2004; Powell *et al.*, 2006; Turkbey *et al.*, 2010). Cardiac structure and diastolic function are altered in obesity, however, in the early stages, systolic function is not affected. These changes appear to be mirrored in people with NAFLD.

There have been no previous studies looking at myocardial strains in people with NAFLD. In healthy ageing, hypertrophic cardiomyopathy and in patients with T2DM, reduced longitudinal shortening is associated with a decrease in circumferential strain and, for the most part, an increase in torsion (Fonseca *et al.*, 2004; Lumens *et al.*, 2006; Cheng *et al.*, 2009; Hollingsworth *et al.*, 2011). In this study, we observed a different pattern: there is evidence of concentric remodelling and reduced longitudinal shortening but endocardial and whole wall circumferential strains were significantly increased and developed faster, without a change in torsion or TSR. The lack of change in torsion is likely due to the fact that endocardial and epicardial strains increased in unison, thus having no effect on the overall level of torsion generated across the myocardial wall. In NAFLD, there appears to be concentric remodelling but without preferential endocardial damage (found in T2DM and ageing), maintaining a normal level of torsion in the myocardium. This means that the inside of the heart wall is able to generate its normal strain levels, and furthermore, the myocardium can cope with the greater strains associated with thicker walls. If subendocardial contractile function is impaired, counteraction of torsion by contraction of subepicardial myofibres is less effective, causing net torsion to increase. Thus TSR increases with impairment of contractile function in the subendocardial layers relative to the subepicardial layers in healthy ageing (Lumens *et al.*, 2006). Although there are changes in cardiac strains in NAFLD and evidence of concentric remodelling, this does not appear to affect the actual ability of the myocardium to generate the necessary contractile forces.

The E/A ratio, a marker of diastolic function, failed to reach statistical significance between the groups and this could represent a type 2 statistical error. However, the NAFLD group demonstrated a 25% decrease compared to controls, with 17/23 NAFLD patients exhibiting values below the mean – 1SEM of the control group. This may be clinically significant and highlights a propensity towards diastolic dysfunction which has also been observed in T2DM and obesity. Early filling percentage, another marker of diastolic dysfunction, was also 10% lower in the NAFLD group and has greater signal to noise than E/A as this was measured directly in our subjects, whereas E/A is determined indirectly from other indices.

There was no significant change in global systolic function in the present patient group, which supports studies looking at cardiac function in obesity and T2DM (Diamant *et al.*, 2003; Scheuermann-Freestone *et al.*, 2003; Vasan, 2003; Wong *et al.*, 2004; Powell *et al.*, 2006; Turkbey *et al.*, 2010). However, the decrease in longitudinal shortening in the NAFLD group is an indicator that the heart's normal movement mechanisms have been disrupted and may eventually lead to a reduction in cardiac output and stroke volume. The reduced shortening ability of the myocardium may have resulted in the increased strains observed as the heart attempted to generate enough mechanical stress to pump as efficiently as possible. EDV was lower in the NAFLD group compared with controls. An increase in EDV increases the preload on the heart and, through the Frank-Starling mechanism (Mangano *et al.*, 1980) of the heart, increases the amount of blood ejected from the ventricle during systole and has a beneficial effect on stroke volume. The decrease in EDV seen in NAFLD may contribute to the lower stroke volume and cardiac outputs observed in our patients, and may have increased the myocardial strain. It may also be an indicator of early stage heart failure.

A retrospective analysis conducted to examine whether the differences shown were due to NAFLD per se and not due to associated obesity, indicates that the cardiac parameters which differ between NAFLD and control are not different merely because the NAFLD group were heavier. It is difficult to ascertain the true impact of NAFLD on cardiac structure and function compared to the effect of blood pressure as the patient group had significantly higher BP, which can lead to LV hypertrophy (Abel *et al.*, 2008). However, BP did not show any significant correlation with the cardiac parameters in the NAFLD group. Use of multivariate statistical analysis could help address concern over hypertension as a potential confounder. Further work should aim to look at comparing patients with NAFLD with BMI matched healthy controls, although this may prove difficult, as increasing obesity levels in the "healthy" population are linked to increasing prevalence of undiagnosed NAFLD (Harrison and Day, 2007). Both the NAFLD group and control group had relatively high fasting glucose levels which could be an indicator of insulin resistance and poor glucose

control. We may then speculate that some of the cardiac changes seen in NAFLD may be attributable to pre-diabetic changes.

In conclusion, significant changes in cardiac structure and function are evident in adults with NAFLD in the apparent absence of cardiac metabolic changes or overt cardiac disease. An important challenge is to identify the earliest manifestations of heart disease with the use of objective surrogate markers of cardiac dysfunction and ultimately aim to prevent overt CVD by initiating earlier therapy. Clinicians should now explore therapies to improve cardiac function as a means to modify the excess risk of CVD associated with NAFLD.

Chapter 6: Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss

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Chapter 6: Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss

6.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum from asymptomatic steatosis to potentially life-threatening non-alcoholic steatohepatitis (NASH) with an overall prevalence of NAFLD in Western countries of 20-30% (Harrison and Day, 2007). Patients with simple steatosis have a relatively benign "liver" prognosis with a 1-2% risk of developing clinical evidence of cirrhosis over 15-20 years. Patients with NASH and fibrosis can progress to cirrhosis at a rate of around 12% over 8 years (Day, 2006). Once cirrhosis develops, patients are at a high risk of developing hepatic decompensation and of dying from a liver-related cause. NAFLD has become a common indication for liver transplant (Erickson, 2008) and is also associated with an increased risk of developing cardiovascular disease and Type 2 diabetes (T2DM) (Targher *et al.*, 2010).

To date there are no proven therapies for treatment of NAFLD other than weight loss, and lifestyle interventions remain the cornerstone of management (Day, 2006; Harrison and Day, 2007). Such interventions have been shown to reduce markers of liver lipid and metabolic control (Kantartzis *et al.*, 2008; St. George *et al.*, 2009; Promrat *et al.*, 2010) in addition to reducing intrahepatic lipid (IHL) (Goodpaster *et al.*, 2003; Oza *et al.*, 2009; Shah *et al.*, 2009; Finucane *et al.*, 2010). Despite this, the weight losses achieved in research trials are not easily replicated in the clinic and are even more difficult to sustain. Consequently there is an urgent need for therapies for the management of NAFLD independent of weight loss.

Physical activity and exercise in NAFLD management could potentially be effective in decreasing IHL. Cross-sectional studies have shown that higher levels of physical activity are associated with lower levels of IHL (Perseghin *et al.*, 2007a; Zelber-Sagi *et al.*, 2008; St. George *et al.*, 2009). Use of aerobic

exercise may not be optimal as, to date, only two studies have reported a beneficial effect of aerobic exercise regimes upon IHL, independent of weight loss (Sreenivasa Baba *et al.*, 2006; Johnson *et al.*, 2009). Population based studies (Zelber-Sagi *et al.*, 2008) suggest that resistance exercise is associated with lower levels of IHL . There have been no previous studies of the direct effect of resistance exercise upon IHL and metabolism, even though it has clear advantages in terms of acceptability and sustainability (Gordon *et al.*, 2009; Larose *et al.*, 2010). Cross-sectional studies (Zelber-Sagi *et al.*, 2008) also suggest that resistance exercise holds the strongest relationship with liver lipid, with higher levels of resistance exercise associated with lower levels of IHL.

The primary aim of this study was to determine the effect of resistance exercise without weight loss, on IHL in adults with NAFLD. The secondary aims were to determine the effect of resistance exercise on mediators of IHL; glucose tolerance and insulin sensitivity, fat oxidation, abdominal adiposity and body composition.

6.2 Subjects and Methods

34 people with NAFLD were invited to be screened for this study. They were recruited from hepatology clinics within the Newcastle upon Tyne Hospitals NHS Foundation Trust or through advertisements in local newspapers. Six of these people subsequently declined taking part, and seven were excluded after screening (two transpired to be taking insulin for their diabetes; one was excluded due to an abdominal hernia; four due to <5% IHL on baseline MRI).

Twenty-one sedentary (≤60 minutes vigorous activity per week) adults with clinically defined non-advanced NAFLD were randomly assigned to either exercise (n=11) or standard care (n=10). Non-advanced NAFLD was defined as >5% IHL and a score of less than -1.445 on the NAFLD Fibrosis Scoring System (Angulo *et al.*, 2007) which indicates a lower percentage chance of having stage 3/4 fibrosis. The study was powered to detect a 2.02% absolute change (delta) in intra-hepatic lipid between the treatment and control groups (SD 2.8%, α 5% and β 50%), based on changes reported from an aerobic exercise study in NAFLD (Johnson *et al.*, 2009). General descriptions can be found in Table 9. Exclusion criteria included: heart or kidney disease; implanted ferrous metal; pre-existing medical conditions preventing participation in the exercise programme; insulin sensitising treatment or dietary change (for people with T2DM, diet and metformin were acceptable for inclusion if stable for six months); and alcohol intake above 21 units for men or 14 units for women. Subjects would be excluded from analysis if body weight changed >2.5% from baseline during the study as this could have an independent effect on IHL. The study protocol was approved by County Durham and Tees Valley 2 Research Ethics Committee. All participants provided written informed consent. Following an initial screening visit, glucose control, lipid oxidation, abdominal lipid depots, and liver lipid were measured at baseline and after the 8-week intervention.

Progressive Exercise Test / Screening Visit: At baseline, a medical history, full physical examination, and progressive exercise test were used to screen for undiagnosed cardiac disease (see Section 2.3 for details).

Physical Activity: Physical activity and energy expenditure were assessed objectively using a validated (St-Onge *et al.*, 2007) multi-sensor array (SenseWear Pro_3, Bodymedia Inc, PA, USA) worn on the right upper arm for seven days prior to randomisation, and for the final seven days of the intervention. The armband provided estimates of: daily energy expenditure; average metabolic equivalents (METs); sedentary time; duration and intensity of physical activity; number of steps; sleep duration; and duration armband worn (see Section 2.5).

Anthropometry: Bodyweight and body composition were measured using an electronic scale and air displacement plethysmography (BodPod, Life Measurement Inc., CA, USA) as described in Section 2.6 (Sardinha *et al.*, 1998; Biaggi *et al.*, 1999; Fields *et al.*, 2005). Height, waist and hip circumference were measured as described in Section 2.3.1.

Liver and Abdominal Fat Measurement: Imaging was undertaken at the Magnetic Resonance Centre at Newcastle University's Campus for Ageing and Vitality, Newcastle upon Tyne. Magnetic resonance studies were performed using a 3.0 Tesla Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands) – see Section 2.7 for details.

Glucose Control: Following an 8 hour overnight fast a cannula was inserted into a forearm vein. A 75g glucose load (Lucozade Original, GlaxoSmithKline, Brentford, UK) was consumed within five minutes. Blood samples were taken at time 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90 and 120 minutes. Samples were analysed as described in Section 2.8 for glucose, insulin and NEFA levels.

Area under the curve (AUC) for the resulting glucose response profile was calculated using the trapezoidal rule (Le Floch *et al.*, 1990) and insulin

resistance determined using the HOMA-IR (see Section 2.8). NEFA suppression (NEFA-S) was assessed during the fsOGTT and the 0-30min change used as a measure of NEFA-S (Patel *et al.*, 2005).

Fasting samples were also analysed in a Clinical Pathology Accredited laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry) for: ALT, total cholesterol, triglycerides and HbA1c - see Section 2.8.

Lipid Oxidation at Rest and Submaximal Exercise: Resting substrate oxidation was determined by expired gas analysis using a Hans Rudolf breathing mask while participants lay supine for 30 minutes in a quiet room. Participants then undertook 60 minutes of submaximal exercise on the recumbent cycle ergometer (details can be found in Section 2.9). Expired air was collected every 15 minutes and respiratory quotient (RQ) was calculated from VO_2 / VCO_2. Substrate oxidation rates and energy expenditure were calculated from oxygen consumption and carbon dioxide production values using stoichiometric equations (Frayn, 1983). Venous blood was collected every 15 minutes during exercise. A further blood sample was taken one hour after the exercise had been completed (the participant remained fasted during this time).

Whole blood glucose was measured immediately after sampling (as detailed in Section 2.8). All other blood samples (NEFA and insulin) were centrifuged, plasma removed then frozen and stored to await batch-analysis. Insulin and NEFA were processed as previously described in Section 2.8.

6.2.1 Study Intervention

Resistance exercise was performed three times per week on non-consecutive days for eight weeks. The programme comprised of eight exercises: biceps curl; calf raise; triceps press; chest press; seated hamstrings curl; shoulder press; leg extension; and lateral pull down (Precor, Woodinville, USA). Each session lasted between 45-60 minutes and consisted of a 10 minute warm-up at approximately 60% maximum heart rate on a cycle ergometer followed by

resistance exercise done as a circuit, ending with a repeat of the warm-up described. The 1 repetition maximum (1RM) was measured (ACSM, 2006) at baseline (which was used to guide the individuals' exercise prescription) and following the intervention. Initially participants did two circuits using 50% of their 1RM, progressing to three circuits, using a minimum 70% of their 1RM, by week 7 (see Appendix 3 for full programme). Participants were encouraged to increase the resistance used each week where possible. Bi-weekly supervised sessions were used to encourage adherence and progression, and to resolve any problems. Heart rate was recorded during each session (Polar RS400, Polar Electro Oy, Finland) and was used alongside exercise logs to assess adherence.

The non-exercising control group continued with standard care and were given the opportunity to receive an exercise prescription at the end of the study.

6.2.2 Statistical Analysis

Following tests for normal distribution, between group differences were evaluated using an unpaired t-test and within group differences using a paired t-test (two-way). Treatment group x time interactions were assessed using a two-way ANOVA. Analyses were performed using Minitab version 15 (Minitab Inc., State College, Pennsylvania). Statistical significance was set at $p<0.05$. Data are mean ± SD unless otherwise stated.

6.3 Results

No subjects withdrew during the trial and all subjects allocated to the exercise group completed all 24 sessions of resistance exercise training. Two subjects (controls) were excluded from analysis (one individual lost >5% of his body weight during the 8 week period and one had a change in his diabetic medication).19 subjects (eight control; 11 exercise) completed the study. The groups were well matched for weight, BMI and waist/hip circumference (Table 9). The exercise group were younger (52 ± 4.0; range 33-72 years) compared with the control group (62 ± 2.6; range 52-71 years; p=0.05). There was no correlation between age and change in IHL (p>0.05).

Anthropometry and Body Composition

BMI remained constant in both groups during the study (32 ± 1.5 to 32 ± 1.4 vs. 32 ± 1.7 to 32 ± 1.5kg.m^2 in exercise and control). There were no significant changes in weight, waist or hip circumference, waist to hip ratio, body composition, visceral or subcutaneous fat in either group (see Tables 9 and 10).

Intrahepatic Lipid

Resistance exercise elicited a 13% relative reduction in IHL with no change in the control (p<0.01; Table 10, Figure 15A). There was a significant time by treatment interaction for resistance exercise (p<0.05; Table 10, Figure 15A). Three of the participants in the exercise group moved from having clinically significant NAFLD to being within normal limits (<5% IHL). No control subject moved into the normal liver fat range.

Blood Lipids and Liver Enzymes

There were no significant changes in blood lipids or ALT in either group (Table 9).

Table 9: Subject Characteristics; values are given as means (SD)

	Control (n= 8)		*Exercise (n=11)*	
	Baseline	**Post-Treatment**	**Baseline**	**Post-Treatment**
Anthropometry				
BMI ($kg \cdot m^{-2}$)	32.3 (1.7)	32.5 (1.5)	32.3 (1.5)	32.3 (1.4)
Weight (kg)	94.0 (4.2)	94.6 (3.8)	96.1 (3.3)	96.1 (3.2)
Waist circumference (cm)	108 (3)	110 (3)	105 (4)	109 (4)
Hip circumference (cm)	110 (2)	115 (3)	110 (3)	111 (3)
Waist : Hip ratio	0.99 (0.01)	0.96 (0.01)	0.96 (0.01)	0.98 (0.01)
VO_{2PEAK} ($mL.kg^{-1}.min^{-1}$)	18.5 (1.8)	-	21.8 (1.2)	-
Metabolic				
ALT ($U.L^{-1}$)	61.6 (14.6)	61.4(15.6)	59.6 (11.7)	59.6 (11.8)
Total cholesterol ($mmol.L^{-1}$)	4.5 (0.4)	4.6(0.3)	5.1 (0.5)	5.0 (0.5)
Triglycerides ($mmol.L^{-1}$)	1.46 (0.2)	1.50 (0.3)	1.79 (0.2)	1.62 (0.2)
HbA1c	6.5 (0.4)	6.8 (0.7)	6.1 (0.2)	6.0 (0.2)

BMI, body mass index; VO_2peak, aerobic capacity; ALT, alanine aminotransferase.

Glucose Control

The exercise group demonstrated improved glucose control after 8 weeks as indicated by a decrease in glucose area under the curve during the fsOGTT compared with control ($p<0.01$; Figure 15B; Table 10). Fasting glucose levels were reduced in the exercise group after the intervention compared with the control but this was not statistically significant (6.0 ± 0.6 to 5.2 ± 0.3 vs. 5.9 ± 0.8 to 6.4 ± 1.0mmol/l; $p=0.086$). Time by treatment interaction for resistance exercise and change in fasting glucose was just outside statistical significance ($p=0.06$). HbA1c remained relatively unchanged in both groups (Table 9).

The exercise group showed a significant improvement in insulin sensitivity after 8 weeks as demonstrated by a decrease in HOMA-IR (5.9 ± 1.8 to 4.6 ± 1.4 vs. 4.7 ± 2.7 to 5.1 ± 1.8; $p<0.05$; Table 10), although time by treatment interaction for resistance exercise and IR failed to show significance ($p=0.055$). Fasting insulin levels remained relatively unchanged in both groups (Table 10).

Table 10: Intrahepatic lipid concentration, subcutaneous and visceral adipose tissue, body composition, glucose control, insulin sensitivity (HOMA-IR), NEFA suppression index, and substrate oxidation during submaximal exercise (RQ). Values are given as means (SD)

	Control (n=8)		***Exercise (n=11)***	
	Baseline	**Post-Treatment**	**Baseline**	**Post-Treatment**
Intrahepatic lipid (%)	11.2 (3.0)	11.5 (2.6)	14.0 (2.8)	12.2 (2.7) * ‡‡
Visceral adipose tissue (cm^2)	2558 (253)	2445 (228)	2098 (244)	2165 (246)
Subcutaneous adipose tissue (cm^2)	3512 (343)	3574 (331)	3275 (351)	3221 (356)
Fat mass (% body mass)	41 (2)	41 (3)	38 (3)	36 (2)
Fasting glucose ($mmol.L^{-1}$)	5.9 (0.8)	6.4 (1.2)	6.0 (0.6)	5.2 (0.3)
Fasting insulin ($p.mol.L^{-1}$)	18.14 (6.41)	18.97 (6.71)	20.55 (6.20)	18.64 (5.62)
Fasting NEFA ($\mu mol.L^{-1}$)	0.48 (0.17)	0.50 (0.18)	0.44 (0.13)	0.43 (0.13)
HOMA-IR	4.7 (1.7)	5.1 (1.8)	5.9 (1.8)	4.6 (1.4)‡
fsOGTT, AUC	839 (106)	940 (149)	885 (81)	777 (56) ** ‡‡
NEFA-S (0-30 min of fsOGTT)	0.07 (0.02)	-0.02 (0.01)	0.01 (0.01)	0.01 (0.01)
Resting RQ	0.86 (0.03)	0.86 (0.02)	0.86 (0.01)	0.86 (0.02)
Sub-maximal exercise RQ	0.90 (0.01)	0.89 (0.01)	0.93 (0.01)	0.91 (0.01) * ‡

HOMA-IR, homeostasis model of insulin resistance; fsOGTT, frequently sampled oral glucose tolerance test; AUC, area under the curve; NEFA-S, non-esterified fatty acid suppression index; RQ, respiratory quotient.

*significant difference treatment x time interaction ($p<0.05$); **significant difference treatment x time interaction ($p<0.01$).
‡significant difference Baseline vs. Post-Treatment ($p<0.05$); ‡‡significant difference Baseline vs. Post-Treatment ($p<0.01$).

NEFA Suppression

Fasting plasma NEFA remained unchanged by the intervention. Similarly, the extent of NEFA suppression during the fsOGTT remained constant in both groups (Table 10).

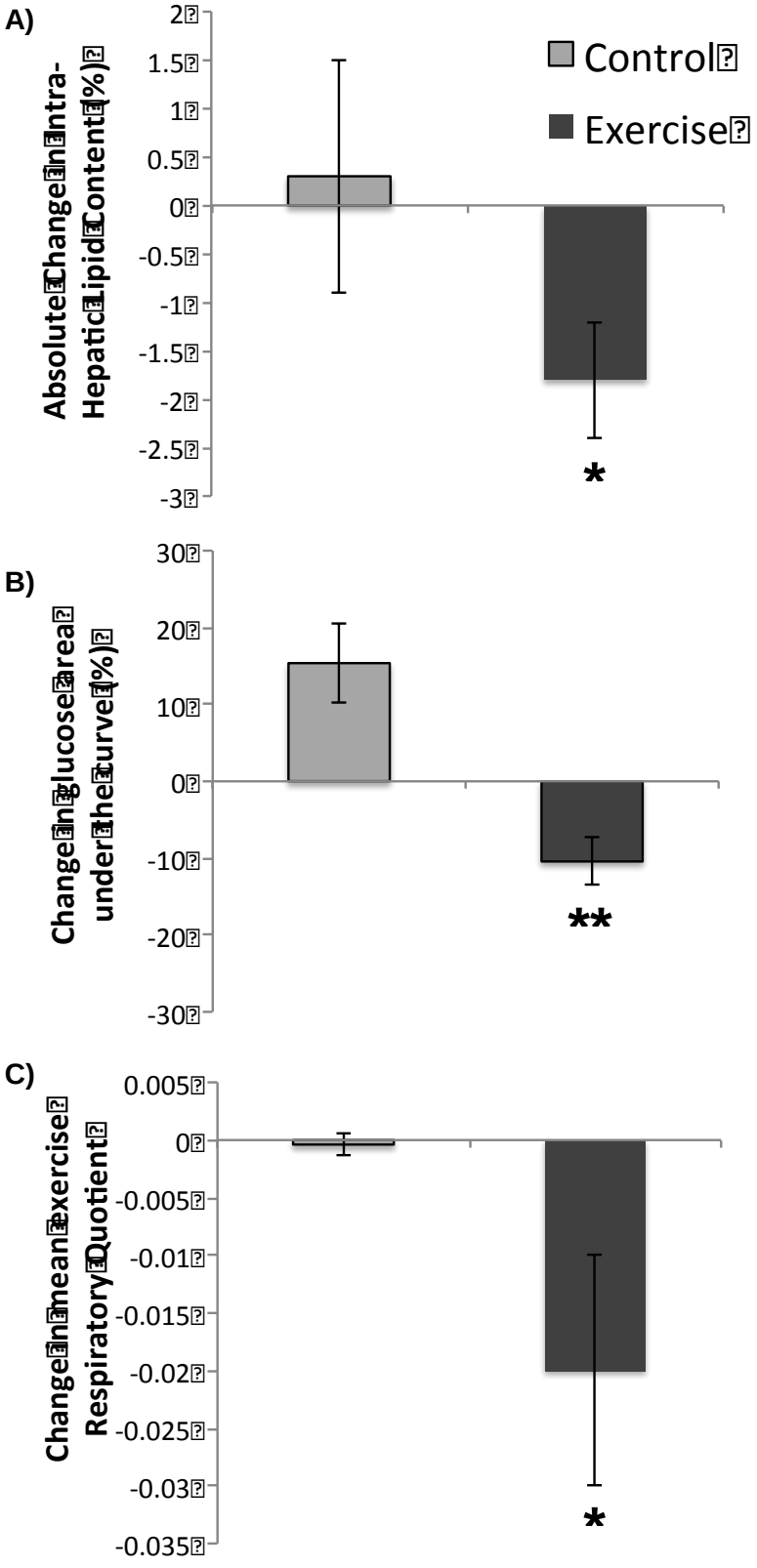
Fat Oxidation

Resistance exercise brought about an increase in fat oxidation during the submaximal exercise test (RQ 0.93 ± 0.0 to 0.90 ± 0.0 vs. 0.90 ± 0.0 to 0.89 ± 0.0, $p<0.05$; $p<0.05$ time by treatment interaction; Table 10; Figure 15C). Resting fat oxidation remained constant in both groups (Table 10).

Physical Activity

There was a wide range of habitual daily activity (number of steps taken daily measured via Sensewear) in both groups at baseline (range 5046 to 12479 exercise, mean = 8492; range 2781 to 9159 control, mean = 5682). The total number of steps taken on a daily basis was significantly higher in the exercise group post intervention when compared with the controls (9848 ± 1113 vs. 5883 ± 1162; p=*0.028*). The change in IHL with both groups combined was weakly associated with the change in number of steps walked per day ($r^2 = 0.28$, $p<0.05$), but not with active energy expenditure ($r^2 = 0.06$, $p>0.05$)

Figure 15: Effect of 8 weeks resistance exercise training (Exercise) or continued standard care (Control) on intrahepatic lipid (A), glucose control from the frequently sampled oral glucose tolerance test (B), and respiratory quotient during submaximal exercise (C); values are means ± SE. * = significantly different from control ($p<0.05$). ** = significantly different from control ($p<0.01$).



6.4 Discussion

This is the first study to examine the effects of resistance exercise on IHL and its mediators in adults with NAFLD. An 8-week resistance exercise programme brought about a ~13% reduction in liver fat. This was accompanied by a ~12% increase in insulin sensitivity, and increased fat oxidation during submaximal exercise in the absence of any change in bodyweight.

Although lifestyle modification combining dietary change and exercise produces a robust reduction in IHL (Harrison and Day, 2007), the data on exercise alone is less definitive. This study demonstrates that resistance exercise is effective in reducing IHL in people with NAFLD. Resistance exercise provides an alternative to aerobic exercise and improves muscular strength, muscle mass and metabolic control safely and effectively in vulnerable populations independent of weight loss (Larose *et al.*, 2010). It places less of a demand on the cardio-respiratory system and may therefore be accessible to more patients (Gordon *et al.*, 2009). Knowledge about the effect of resistance exercise upon IHL and metabolic control, independent of weight loss, will assist clinical care teams in their advice for people with NAFLD. All participants completed the 8-week programme, showing good adherence and tolerance.

To date, only two studies have reported the effect of exercise alone on liver health in adults with NAFLD, both having used aerobic exercise but only one reports a direct measure of IHL. A 4-week aerobic exercise intervention showed a similar absolute reduction in IHL (^{1}H-MRS) as seen during the present study. The aerobic exercise brought about a reduction in IHL from 8.6 to 6.8% (Johnson *et al.*, 2009). A 12-week intervention reported a 47% (47 U/L) and 48% (30 U/L) reduction in ALT and AST respectively (Sreenivasa Baba *et al.*, 2006). However, these biomarkers can be elevated in the absence of excess liver fat and within the normal in ranges in the presence of elevated liver fat making them poor indicators for actual liver fat (Mofrad *et al.*, 2003; Szczepaniak *et al.*, 2005; Fracanzani *et al.*, 2008). Although the changes in

liver lipid following exercise therapy are significant, the absolute change (~2% IHL) was modest compared to the ~10% reduction reported after an 8kg weight loss from caloric restriction (Petersen *et al.*, 2005). It should also be considered that in this study, the exercise group had higher IHL at baseline (14%) than the control group (12%) and whether this may influence subsequent IHL response to exercise (i.e. the more liver fat there is at baseline, the more there is to lose). Therefore, the clinical value of exercise appears likely to be as an adjunct to caloric restriction.

The observed improvement in glucose control is consistent with findings in populations with impaired glucose control or T2DM (Strasser *et al.*, 2010; Zanuso *et al.*, 2010). The aerobic exercise studies in NAFLD have either reported no change (Johnson *et al.*, 2009) or not reported measures of glucose control (Sreenivasa Baba *et al.*, 2006). Most studies in impaired glucose control or T2DM have used liver function tests, particularly ALT and AST levels, or blood lipid levels as surrogate markers for IHL (Sigal *et al.*, 2007; Gordon *et al.*, 2009) again making extrapolation to IHL difficult.

We observed a pure exercise effect on IHL which did not involve any change in visceral fat in the patients. There is increasing evidence that the two depots are not mechanistically linked but both tend to reflect adiposity (Ravikumar *et al.*, 2008). Recent findings from the Framingham Heart Study (Speliotes *et al.*, 2010), and a much smaller cohort (Hoenig *et al.*, 2010), show IHL to be associated with dyslipidemia and dysglycaemia independently of visceral fat. Our observation of decreased IHL in the absence of any observable change in visceral or subcutaneous fat provides further information on the separate regulation of IHL and visceral fat.

The mechanisms underlying the change in IHL following exercise are likely to reflect changes in energy balance, circulatory lipids, and insulin sensitivity. Insulin sensitivity plays a significant role in liver lipid homeostasis. High levels of circulatory insulin up-regulate SREBP-1c and ChREBP expression in the liver (Tamura *et al.*, 2005; Lavoie and Gauthier, 2006), stimulating *de-novo*

lipogenesis and increasing IHL. In healthy normoglycaemic humans, hepatic *de novo* lipogenesis contributes approximately 5% and 18-23% of IHL in the fasted and postprandial states, respectively (Timlin and Parks, 2005). Whereas *de novo* lipogenesis is constantly elevated in those with NAFLD contributing approximately 26% of IHL irrespective of feeding state (Donnelly and Smith, 2005). Elevated circulating triglycerides exacerbate this problem by impeding insulin stimulated glucose uptake (Ferrannini *et al.*, 1983). Thus creating a vicious cycle where elevated IHL levels impede hepatic insulin action, causing increased portal insulin levels and further increasing IHL (Taylor, 2008).

The findings suggest that the introduction of resistance exercise breaks this cycle by improving glucose control and fat oxidation. Our observations would support other reports that resistance exercise increases whole-body glucose disposal (Ferrara *et al.*, 2006) at least partly due to increases in skeletal muscle GLUT4, glycogen synthase expression and activity, insulin receptor, and glycogen storage (Holten *et al.*, 2004). Thus skeletal muscle in the resistance exercising individual can act to safely sequester circulating fatty acids and glucose, reducing the impact of insulin stimulated *de novo* lipogenesis in the liver. Aerobic exercise has been shown to increase intra-myocellular triglyceride synthesis (Pruchnic *et al.*, 2004; Dube *et al.*, 2008), while decreasing accumulation of fatty acid metabolites and suppressing the proinflammatory state associated with insulin resistance (Schenk and Horowitz, 2007). It remains to be determined whether exercise has any direct transcriptional effects on the liver.

In conclusion, well-tolerated resistance exercise reduced IHL, increased insulin sensitivity, and improved metabolic flexibility in NAFLD independent of weight loss. The absolute effect of resistance exercise studied in isolation was modest but similar to that of aerobic exercise. The benefits of resistance exercise combined with caloric restriction in the clinical management of NAFLD will depend upon long-term maintenance and sustainability of exercise - this now needs to be investigated.

Chapter 7: General Discussion

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Chapter 7: General Discussion

Metabolic disease remains a growing problem in Western countries, with epidemic turning into pandemic. Yet despite this, we are continuing to live longer, exposing individuals, their immediate support and society in general to the burden of managing chronic disease. There is a close relationship between energy expenditure and metabolic control and this plays the most significant role in the development of metabolic disease. When an imbalance occurs between the energy taken in, in terms of diet, and the energy out, in terms of physical activity or exercise, the liver, heart, muscle, and adipose tissue are all affected. The impact of low levels of physical activity and lack of exercise affects all of these organs and also cross-organ communication, creating a vicious cycle. For example, a decrease in energy expenditure, as a result of inactivity or lack of physical activity or exercise, can result in weight gain, and in particular an increase in adipose tissue mass. Physical inactivity also has a direct impact on muscle, reducing its capacity to oxidise fat and store glucose. The enlarged mass of adipose tissue and reduced ability to oxidise fat results in greater levels of circulatory fatty acids which arrive at the liver via the portal vein or the heart via the vena cava. This increase in fat delivery can lead to ectopic fat storage in both organs, resulting in NAFLD and cardiac steatosis respectively. The present data also extends to demonstrate changes in cardiac function in people with NAFLD in the absence of overt cardiac disease. Since CVD is the second biggest cause of mortality in people with NAFLD, if the changes can be managed and/or reversed in this early stage, some of the later cardiac complications may be prevented.

Exercise and physical activity break this cycle by reducing adipose tissue and improving the ability of muscle to oxidise fat. This increase in fatty acid oxidation lowers circulating fatty acids, reducing the exposure of other organs to excess fat. As a result, physical activity and exercise not only improve the metabolic and functional capacity of individual tissues, but also have a combined effect on the system as a whole; conferring a sustained benefit beyond the immediate exercise or movement stimulus.

Current clinical practice recommends the use of lifestyle interventions in the treatment of NAFLD (Harrison and Day, 2007). These interventions incorporate weight loss, diet and physical activity, however the optimal “dose” of each component is unknown, and clinicians often place the emphasis on weight loss and dietary change, rather than challenging physical activity habits. However, the evidence upon which these guidelines were based is limited and need improving in order to provide informed guidance and options to people with NAFLD. This is particularly important as, although weight loss remains the mainstay of clinical management of NAFLD, it is exceptionally difficult to achieve and even more difficult to sustain in the free-living environment. As a result, the use of exercise / physical activity may provide additional therapeutic avenues to people where weight loss is unachievable. The research within this thesis begins to provide the evidence upon which physical activity and exercise can be used effectively in the clinical care of people with NAFLD.

This research shows that there is a general decrease in physical activity levels in people with NAFLD, coupled with an increase in inactivity. These alterations in activity habits, in part, reflect that of the general population as people worldwide, particularly in Western countries, are undertaking less physical activity and becoming more sedentary (Blair, 2009). The growing levels of physical inactivity are playing a major role in the increasing prevalence of obesity, T2DM and NAFLD. Finding ways to target the consequences of physical inactivity are one of the greatest challenges of our generation. Historically, aerobic exercise interventions have been applied to understand the effect of exercise upon metabolic health and wellbeing. Although these studies provided a useful insight into the use of exercise as a therapy, aerobic exercise is, in general, poorly tolerated by patients who as a group have low levels of physical fitness. Other approaches and options are necessary. This thesis challenged this by demonstrating that resistance exercise therapy was successful in reducing liver fat and conferred positive benefits on the mediators of liver fat. The resistance exercise was well tolerated by the patients involved and may prove to be a popular alternative to aerobic exercise with some of our

patient group that have other comorbidities. However, the results of this study should be taken in perspective. Compared to weight loss studies (Kirk *et al.*, 2009; Promrat *et al.*, 2010), the absolute change in liver fat was moderate, but comparable to that achieved with aerobic exercise alone (Johnson *et al.*, 2009; van der Heijden *et al.*, 2009).

Clinically, lifestyle interventions should still be the main over-arching therapy for patients with uncomplicated NAFLD, however more emphasis should be placed on increasing day-to-day activity levels and decreasing sedentary time. Even without weight loss or dietary change, small, achievable changes in activity habits could hold benefits for overall metabolic control, and more specifically liver and cardiac health. Programmes that encourage objective self-monitoring of activity levels may improve patient motivation and promote long-term behaviour change. In NAFLD physical activity guidelines are limited, but it seems that the more we can encourage people with NAFLD to move and the less time to spend sitting, the more positive impact there is on metabolic health. For added health benefits, exercise should also be incorporated as a regular part of the weekly routine. Further large scale studies are needed to ascertain the optimal type, frequency, duration and intensity of exercise that would be of most benefit to prevent NAFLD or to stop disease progression.

One factor that may help stem the NAFLD epidemic is an increased public awareness of the condition. Most people in the general population have not heard of NAFLD and many link liver diseases to excess alcohol consumption, with its associated stigmas. Highlighting NAFLD as a lifestyle related disease, which in most cases is reversible if tackled early enough, may improve treatment outcomes. Many patients with NAFLD remain asymptomatic, unaware of their diagnosis and as such are oblivious to the ramifications excess liver fat may have on long-term health. This lack of understanding of the risks associated with NAFLD, and how it can be potentially treated, minimises the incentive for the individual to make any lifestyle changes. Most of the patients with NAFLD are managed within Primary Care by general practitioners and

practice nurses. The results of studies looking at NAFLD management and treatment, including those from this report, need to be filtered through to the healthcare practitioners looking after these people on a day-to-day basis. Exercise on Referral schemes should be considered to offer these patients guidance on exercise and how to get started, in a safe and controlled environment. The equivalent of cardiac rehabilitation classes could be introduced to target people with NAFLD at an early stage, to get them moving and hopefully prevent disease complications such as T2DM and CV disease. Currently, these services are limited within the UK and are not necessarily at the forefront of the doctors' minds when seeing these people in clinic. Managing people at an early stage, empowering them to take control of their own lifestyle and offering ways of helping them to achieve these changes in the long-term will have lasting health benefits for the individual, but will also save the NHS vast amounts of money in future healthcare costs.

7.1 Future directions

Although the data contained within this thesis provides a robust base upon which recommendations around physical activity, exercise, and NAFLD can begin to be made, there remain a large number of unanswered questions. There are two main physiological questions that need to be answered: 1) what is the interaction between weight loss, exercise and liver fat? and 2) what is the dose response relationship of different forms of exercise with liver fat? It is clear that weight loss produces very significant and rapid changes in liver fat. The impact of exercise, in turn, is modest. However, the benefits of weight loss are temporary with most people regaining weight after that initial loss. Exercise therapy helps prevent weight regain. As a result, the most clinically effective position for exercise therapy may indeed be following weight loss and in preventing weight regain. An understanding of the 'dose' of exercise therapy required to effectively change liver fat and its mediators will assist patients and clinical care teams understand what the minimal and optimal amount of exercise required to improve liver health. Furthermore, over the past two decades, our knowledge about the molecular adaptations of skeletal muscle to

exercise has driven the use of exercise as a clinical therapy in conditions such as T2DM. Studies which begin to characterise the changes in liver signalling with exercise hold the potential to drive the use of exercise as an effective therapy in liver disease.

7.2 Conclusion

The work contained within this thesis demonstrates that low levels of physical activity are prominent in people with NAFLD and that targeting this with resistance exercise therapy confers benefits to both liver lipid and the factors promoting its accumulation. Over the duration of the work described in this thesis, the number of studies reporting on exercise and liver fat in people with NAFLD has increased markedly. The new information contained within this thesis contributes to this body of knowledge and, over time, will improve the management of a condition that is an increasing burden to the people of the Western world.

Chapter 8: Appendices

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Appendix 1: Consent Forms

Consent Form for Healthy Volunteers for the Physical Activity Study

Newcastle Magnetic Resonance Centre
Campus for Ageing and Vitality
Newcastle General Hospital
Westgate Road
Newcastle upon Tyne
NE4 6BE
T: +44 (0)191 256 3691

Identification number for this trial:

CONSENT FORM

Title of Project: Evaluation of everyday metabolism and energy expenditure in healthy people.

Name of researchers: Miss Kate Hallsworth and Dr Michael Trenell

Please initial box

1. I confirm that I have read and understand the information sheet dated May 2009 (version 1-2) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation 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 take part in the above study. ☐

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

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

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Patient Identification number for this trial:

CONSENT FORM

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

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

Please initial box

4. I confirm that I have read and understand the information sheet dated January 2011 (version 4) for the above study and have had the opportunity to ask questions. ☐

5. 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. ☐

6. I agree to my GP being informed of my participation in the study ☐

7. I agree to take part in the above study. ☐

5. I agree to take part in the Sensewear validation sub-study ☐

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

Consent Form for the Resistance Exercise Study

MRC Muscle Performance and Training Laboratory
Campus for Ageing and Vitality
Newcastle General Hospital
Westgate Road
Newcastle upon Tyne
NE4 6BE
T: +44 (0)191 248 1150

Patient Identification number for this trial:

CONSENT FORM

Title of Project: Resistance exercise and non-alcoholic fatty liver disease

Name of researchers: Dr M Trenell, Kate Hallsworth, 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 March 2009 (version 1-1) 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. ☐

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 2: Questionnaires

Physical Activity Readiness Questionnaire (PARQ)

Name: _____ **Date of Birth:** _____

		Please choose	
1	Has your doctor ever said that you have a *heart condition* and that you should only do physical activity recommended by a doctor?	YES	NO
2	Do you ever feel *pain* 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 faint or have spells of dizziness?	YES	NO
5	Do you have a *joint problem* (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	Are you currently taking any *medication*? 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?	YES	NO
10	How many times a week do you 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: _____

Magnetic Resonance Patient Safety Screening Questionnaire

Patient name:_____Date of birth_____ / ____ / ____

Please check the following carefully. These items can interfere with MR examinations, and some may be hazardous to your safety.

Any queries contact the Radiographers on 0191 2563691 Ext. 21105

Have you had any operations on your **HEAD**?	Yes	No
Have you had any operations on your **SPINE**?	Yes	No
Have you had any operations on your **CHEST or HEART**?	Yes	No
Have you had any operations involving the use of **METALLIC CLIPS, PINS or PLATES**?	Yes	No

Do you have a Cardiac Pacemaker?	Yes	No
Do you have an **Aneurysm Clip**?	Yes	No
Do you have a Cochlear implant?	Yes	No

Have you ever worked with metal?	Yes	No
Is there any possibility that you could have **metal in your eye**?	Yes	No
Have you ever had a shrapnel or bullet injury?	Yes	No

Are you wearing?

Dentures with metal	Yes	No
A hearing aid	Yes	No
An artificial limb	Yes	No
Body piercing/jewelry	Yes	No
A CARDIAC / HRT / NICOTINE Patch	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

FOR WOMEN OF CHILDBEARING AGE: Could you be pregnant?	Yes	No

ALL metal worn or carried on your person is to be removed.

Pens, Spectacles, Keys, Money, Jewellery, Watches, Hairgrips, Dentures, Scissors, Credit Cards, Hearing Aids, Bra, Belt, Surgical Supports, etc.

Do not sign this form yet. A member of the Centre staff will go through the form with you and explain the MRI scan procedure.
I understand the procedure of a MRI examination. I also understand the above questions.

Patient's Signature: _____ Date: _____

Staff Signature: _____Date: _____

International Physical Activity Questionnaire (IPAQ)

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** and **moderate** 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. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No → **Skip to PART 2: TRANSPORTATION**

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ hours per day

_____ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ days per week

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ hours per day

_____ minutes per day

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ days per week

☐ No job-related walking → **Skip to PART 2: TRANSPORTATION**

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ hours per day

_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ days per week

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ hours per day

_____ minutes per day

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ days per week

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ hours per day

_____ minutes per day

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ days per week

☐ No walking from place to place → **Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY**

13. How much time did you usually spend on one of those days walking from place to place?

_____ hours per day

_____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ days per week

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ hours per day

_____ minutes per day

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ days per week

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ hours per day

_____ minutes per day

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ days per week

☐ No moderate activity inside home → **Skip to PART 4: RECREATION,SPORT AND LEISURE-TIME PHYSICAL ACTIVITY**

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ hours per day

_____ minutes per day

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ days per week

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ hours per day

_____ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ days per week

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ hours per day

_____ minutes per day

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ days per week

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ hours per day

_____ minutes per day

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while 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. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ hours per day

_____ minutes per day

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

_____ hours per day

_____ minutes per day

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

Appendix 3: Resistance Exercise Programme

Induction

Volunteer's Name: Date:

Swipe card provided	☐
Orientation to the building	☐
Emergency procedures	☐
Provide University induction booklet and opening times	☐
Teach warm up/down	☐
Explain re: breathing techniques, speed of movement	☐

Work out 1RM (kg) for each exercise
(Check technique on each exercise)

1. Biceps _____
2. Calf Raise _____
3. Triceps _____
4. Chest Press _____
5. Hamstrings _____
6. Shoulder Press _____
7. Quadriceps _____
8. Lat. Pull Down _____

Comments:

Week 1 – 50% of 1RM; 2 Circuits **Body Weight (kg):**

Session 1- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		8		
2. Calf Raise		8		
3. Triceps		8		
4. Chest Press		8		
5. Hamstrings		8		
6. Shoulder Press		8		
7. Quadriceps		8		
8. Lat. Pull Down		8		

Any comments about the session?

Session 2- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		10		
2. Calf Raise		10		
3. Triceps		10		
4. Chest Press		10		
5. Hamstrings		10		
6. Shoulder Press		10		
7. Quadriceps		10		
8. Lat. Pull Down		10		

Any comments about the session?

Session 3- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		12		
2. Calf Raise		12		
3. Triceps		12		
4. Chest Press		12		
5. Hamstrings		12		
6. Shoulder Press		12		
7. Quadriceps		12		
8. Lat. Pull Down		12		

Any comments about the session?

Week 2 – 50% of 1RM; 2 Circuits **Body Weight (kg):**

Session 1- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		8		
2. Calf Raise		8		
3. Triceps		8		
4. Chest Press		8		
5. Hamstrings		8		
6. Shoulder Press		8		
7. Quadriceps		8		
8. Lat. Pull Down		8		

Any comments about the session?

Session 2- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		10		
2. Calf Raise		10		
3. Triceps		10		
4. Chest Press		10		
5. Hamstrings		10		
6. Shoulder Press		10		
7. Quadriceps		10		
8. Lat. Pull Down		10		

Any comments about the session?

Session 3- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		12		
2. Calf Raise		12		
3. Triceps		12		
4. Chest Press		12		
5. Hamstrings		12		
6. Shoulder Press		12		
7. Quadriceps		12		
8. Lat. Pull Down		12		

Any comments about the session?

Week 3– 60% of 1RM; 2 Circuits **Body Weight (kg):**

Session 1- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		8		
2. Calf Raise		8		
3. Triceps		8		
4. Chest Press		8		
5. Hamstrings		8		
6. Shoulder Press		8		
7. Quadriceps		8		
8. Lat. Pull Down		8		

Any comments about the session?

Session 2- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		10		
2. Calf Raise		10		
3. Triceps		10		
4. Chest Press		10		
5. Hamstrings		10		
6. Shoulder Press		10		
7. Quadriceps		10		
8. Lat. Pull Down		10		

Any comments about the session?

Session 3- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		12		
2. Calf Raise		12		
3. Triceps		12		
4. Chest Press		12		
5. Hamstrings		12		
6. Shoulder Press		12		
7. Quadriceps		12		
8. Lat. Pull Down		12		

Any comments about the session?

Week 4 – 60% of 1RM; 2 Circuits **Body Weight (kg):**

Session 1- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		8		
2. Calf Raise		8		
3. Triceps		8		
4. Chest Press		8		
5. Hamstrings		8		
6. Shoulder Press		8		
7. Quadriceps		8		
8. Lat. Pull Down		8		

Any comments about the session?

Session 2- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		10		
2. Calf Raise		10		
3. Triceps		10		
4. Chest Press		10		
5. Hamstrings		10		
6. Shoulder Press		10		
7. Quadriceps		10		
8. Lat. Pull Down		10		

Any comments about the session?

Session 3- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2
1. Biceps		12		
2. Calf Raise		12		
3. Triceps		12		
4. Chest Press		12		
5. Hamstrings		12		
6. Shoulder Press		12		
7. Quadriceps		12		
8. Lat. Pull Down		12		

Any comments about the session?

Week 5 – 60% of 1RM; 3 Circuits **Weight (kg):**

Session 1- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		8			
2. Calf Raise		8			
3. Triceps		8			
4. Chest Press		8			
5. Hamstrings		8			
6. Shoulder Press		8			
7. Quadriceps		8			
8. Lat. Pull Down		8			

Any comments about the session?

Session 2- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		10			
2. Calf Raise		10			
3. Triceps		10			
4. Chest Press		10			
5. Hamstrings		10			
6. Shoulder Press		10			
7. Quadriceps		10			
8. Lat. Pull Down		10			

Any comments about the session?

Session 3- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		12			
2. Calf Raise		12			
3. Triceps		12			
4. Chest Press		12			
5. Hamstrings		12			
6. Shoulder Press		12			
7. Quadriceps		12			
8. Lat. Pull Down		12			

Any comments about the session?

Week 6– 60% of 1RM; 3 Circuits **Body Weight (kg):**

Session 1- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		8			
2. Calf Raise		8			
3. Triceps		8			
4. Chest Press		8			
5. Hamstrings		8			
6. Shoulder Press		8			
7. Quadriceps		8			
8. Lat. Pull Down		8			

Any comments about the session?

Session 2- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		10			
2. Calf Raise		10			
3. Triceps		10			
4. Chest Press		10			
5. Hamstrings		10			
6. Shoulder Press		10			
7. Quadriceps		10			
8. Lat. Pull Down		10			

Any comments about the session?

Session 3- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		12			
2. Calf Raise		12			
3. Triceps		12			
4. Chest Press		12			
5. Hamstrings		12			
6. Shoulder Press		12			
7. Quadriceps		12			
8. Lat. Pull Down		12			

Any comments about the session?

Week 7 – 70% of 1RM; 3 Circuits **Body Weight (kg):**

Session 1- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		8			
2. Calf Raise		8			
3. Triceps		8			
4. Chest Press		8			
5. Hamstrings		8			
6. Shoulder Press		8			
7. Quadriceps		8			
8. Lat. Pull Down		8			

Any comments about the session?

Session 2- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		10			
2. Calf Raise		10			
3. Triceps		10			
4. Chest Press		10			
5. Hamstrings		10			
6. Shoulder Press		10			
7. Quadriceps		10			
8. Lat. Pull Down		10			

Any comments about the session?

Session 3- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		12			
2. Calf Raise		12			
3. Triceps		12			
4. Chest Press		12			
5. Hamstrings		12			
6. Shoulder Press		12			
7. Quadriceps		12			
8. Lat. Pull Down		12			

Any comments about the session?

Week 8– 70% of 1RM; 3 Circuits **Body Weight (kg):**

Session 1- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		8			
2. Calf Raise		8			
3. Triceps		8			
4. Chest Press		8			
5. Hamstrings		8			
6. Shoulder Press		8			
7. Quadriceps		8			
8. Lat. Pull Down		8			

Any comments about the session?

Session 2- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		10			
2. Calf Raise		10			
3. Triceps		10			
4. Chest Press		10			
5. Hamstrings		10			
6. Shoulder Press		10			
7. Quadriceps		10			
8. Lat. Pull Down		10			

Any comments about the session?

Session 3- Date:

Exercise	Weight	Number of Reps.	Number Completed Circuit 1	Number Completed Circuit 2	Number Completed Circuit 3
1. Biceps		12			
2. Calf Raise		12			
3. Triceps		12			
4. Chest Press		12			
5. Hamstrings		12			
6. Shoulder Press		12			
7. Quadriceps		12			
8. Lat. Pull Down		12			

Any comments about the session?

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