Exercise, Glucose Control, and Liver Fat: Providing the Evidence for Translation into Clinical Care

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Abstract

Non-alcoholic fatty liver disease (NAFLD) has become the most common form of liver disease throughout much of the World. It affects between one in five and one in three adults in the general population. It is now believed to be the leading cause of liver cirrhosis and hepatocellular carcinoma. However, the majority of people with NAFLD do not go on to develop terminal liver disease but instead have an uncertain prognosis that can often include type 2 diabetes, cardiovascular disease, and/or non-hepatic cancers. Indeed, NAFLD is frequently accompanied impaired glucose control, and almost always suboptimal insulin sensitivity.

This thesis explores the only currently recommended therapy – weight reduction by lifestyle modification. It reviews the published evidence supporting this recommendation by applying a systematic approach to review the literature, but examines the findings in the broader context of common NAFLD comorbidities and sequelae. It also examines interaction of age and physical activity with liver fat, specifically in women, using both primary and secondary research methods. Finally, it explores exercise, particularly high-intensity intermittent training as a means to reduce liver fat, improve body composition, and attenuate insulin resistance independent of weight change and dietary advice.

The principle finding is that, although the literature supports the recommendation of weight reduction, exercise can be an effective therapy to reduce liver fat and improve glucose control/insulin independent of weight change in adults with NAFLD. High-intensity intermittent training is particularly

effective for liver fat reduction, improves glucose control/insulin resistance, and results in positive changes to body composition.

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In Text Acronyms

^{1}H-MRS	proton magnetic resonance spectroscopy
ALT	alanine aminotransferase
AMARES	non-linear least squares algorithm
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AST	aspartate aminotransferase
AUC	area under the curve
BMI	body mass index
CMIE	continuous moderate intensity exercise
CPA	Clinical Pathology Accredited
CT	computed tomography
CV	coefficient of variation
DAG	diacylglycerol
DPS	diabetes prevention study/studies
ECG	electrocardiogram
EGIR	European Group for the Study of Insulin Resistance
FABP	plasma membrane-associated fatty acid-binding protein
FAT/CD36	fatty acid translocase/cluster of differentiation 36
FATP	fatty acid transport proteins
FFM	fat free mass
g	grams
GGT	γ-glutamyl transferase
GLUT	glucose transporter
HbA_{1c}	glycated haemoglobin A_{1c}
HDL	high density lipoprotein
HIIT	high intensity intermittent training
HOMA-IR	homeostatic model of insulin resistance
HOMA-β	homeostatic model β-cell function
HR	heart rate (as beats per minute)
HR_{max}	maximal heart rate
HR_{peak}	peak heart rate
IDL	intermediate density lipoprotein
IHTAG	intrahepatic triacylglycerol
kcal	kilocalories
kg	kilogram
kHz	kilohertz
L	litre
LDL	low density lipoprotein
mL	millilitres
mm	millimetres
MRI	magnetic resonance imaging
NAFLD	non-alcoholic fatty liver disease
NAS	Non-alcoholic fatty liver disease histological activity score
NASH	non-alcoholic steatohepatitis
NCEP	National Cholesterol Education Program
NEFA	non-esterified fatty acids
NHANES	National Health and Nutrition Examination Survey
p	p-value

r	correlation coefficient
RER	respiratory exchange ratio
RMR	resting metabolic rate
RNA	ribonucleic acid
RPE	rate of perceived exertion
SGLT	sodium glucose transporter
SIT	sprint interval training
T	Tesla
TAG	triacylglycerol
US	United States
VCO_2	volume of carbon dioxide
VLDL	very low density lipoprotein
VO_2	volume of oxygen
VO_{2max}	volume of oxygen at maximum aerobic exercise intensity
VO_{2peak}	volume of oxygen at peak aerobic exercise intensity
$VO_{2peakFFM}$	peak volume of oxygen per kilogram of fat free mass
VOXEL	volume element

Chapter 1. Introduction and Literature Review

1.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of conditions ranging from simple hepatic steatosis through to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis [1]. The condition is closely associated with, and arguably a component of, metabolic syndrome [2], especially insulin resistance and type 2 diabetes [3,4]. NAFLD is estimated to affect 20-30 percent of adults [5] and approximately 3% of children [6]. The prevalence of NAFLD is increases in obese populations with estimates as high as 85% in morbidly obese adults [5], and 77% in obese children [6]. In addition to obesity, predictors of NAFLD include physical inactivity [7], and low cardiorespiratory fitness [7,8]. As the prevalence of both obesity and sedentariness continue to grow, albeit not as fast as in previous decades [9,10], NAFLD is likely to become even more common in the foreseeable future. Indeed, as some of the existing prevalence data are over 10 years old [5], the problem is already likely to be more extensive than published estimates suggest.

The costs associated with NAFLD specifically are difficult to quantify given its close association with metabolic syndrome and type 2 diabetes, which themselves are causally linked to cardiovascular disease, neuropathy, and nephropathy [11]. However, type 2 diabetes alone costs the UK National Health Service approximately £8.8 billion in direct costs and a further £13 billion in indirect costs; this is estimated to increase to £15 billion and £20 billion, respectively, by the year 2035 [12]. Much of this cost may be avoidable if NAFLD is prevented or appropriately managed in its early stages and therefore removed as a contributing factor to the development of type 2 diabetes.

There are presently no approved pharmaceutical protocols specifically for the treatment of NAFLD, therefore lifestyle modification with the goal of weight reduction is the only recommended therapy for patients with NAFLD [13,14]. Research has shown that weight reduction, changes in diet, and exercise can all help to reduce intrahepatic triacylglycerol (IHTAG) concentration and

increase insulin sensitivity, but the approach with the greatest effectiveness remains to be determined [15].

This chapter provides a broader context for the primary and secondary research presented in subsequent chapters. As this thesis focuses on the effects of exercise on NAFLD in adults, data from paediatric populations will not be reviewed.

This thesis addresses the following questions through secondary research:

1. *What is the efficacy of lifestyle interventions in adults with NAFLD in terms of* (see *Chapter 2*):

- Reducing IHTAG and/or liver aminotransferases;
- Improving histological parameters; and
- Improving glucose control/insulin sensitivity.

2. *Is high intensity intermittent training a feasible option for patients with NAFLD?*

and seeks to address the following questions via primary research:

3. *What is the relationship between ageing and physical activity, and fitness, body composition, glucose control, and liver fat?*

4. *What is the effect of high intensity intermittent training in patients with NAFLD on:*

- intrahepatic triacylglycerol;
- glucose control and insulin sensitivity;
- substrate utilisation at rest and during moderate intensity activity;
- body composition;
- blood pressure; and
- biomarkers of cardiovascular and liver disease risk

1.2 Normal Macronutrient Metabolism

Metabolic syndrome, type 2 diabetes, and NAFLD result predominantly from lifestyle factors; those relating to diet and physical activity being the most broadly studied (see *Section 1.7* for further discussion). All three conditions are marked by abnormalities in nutrient metabolism in both the fasted and fed, i.e. postprandial, states [16,17]. The latter being the state in which most people not deliberately fasting spend the majority of the time; partly due to the ready availability of food and opportunities to consume it [18]. An insight into normal and altered postprandial metabolism thus provides essential context for understanding NAFLD and commonly related metabolic diseases.

The postprandial state is characterised by an array of changes in host physiology to facilitate the digestion, absorption, and systemic distribution of the nutrients consumed. Anticipation of food leads to increased production of saliva [19], excretion of gastric juices [18], and changes in blood hormone concentrations [20]. There are marked differences in physiological responses to a meal between those with metabolic diseases such as NAFLD and type 2 diabetes, and those with ‘normal’ metabolism [21-24], and between those recently sedentary or active [25-28]. The bulk of what is known about postprandial metabolism in metabolic conditions relates to carbohydrates and lipids.

1.2.1. Carbohydrates

Dietary carbohydrates come in two distinct forms, those joined by α 1-4 and α 1-6 glycosidic linkages and therefore able to be digested by human enzymes and absorbed in the form of the monosaccharides glucose, fructose, or galactose, and those resistant to human enzymes and hence absorption, often referred to as dietary fibre [29].

Digestion of dietary carbohydrates begins in the mouth with the release of salivary α-amylase, but actual enzymatic digestion takes place in the stomach and small intestine where pancreatic α-amylase and a host of glycosidases are also present [18]. Absorptions takes place predominantly in the small intestine once polysaccharides have been broken down into their respective monomers. Glucose is absorbed via the sodium-glucose transporter (SGLT) 1, 2, and 4,

and the glucose transporter (GLUT) 2 [30-32]. Galactose is absorbed by SGLT1, and fructose by GLUT5 [30].

Absorbed monosaccharides enter the hepatic portal circulation and thus can undergo first pass metabolism. In the case of fructose and galactose hepatic absorption is virtually complete [18]. Hepatic glucose absorption is less complete with the 'direct pathway' of glucose conversion to glycogen making only a modest contribution to liver glycogen [33,34], which is predominantly synthesised from 3-carbon outputs of gluconeogenesis, e.g. lactate, glycerol, and gluconeogenic amino acids, via the 'indirect pathway' [34]. Glycogenesis and gluconeogenesis are processes undergoing a continuous cycling in both the postprandial and fasted state; net glycogenesis however, appears to be very sensitive to glucose availability and is down-regulated in the presence of a large glucose supply [34].

Fructose and galactose absorbed by the liver are converted to fructose-1-phosphate and galactose-1-phosphate, respectively [18]. Thereafter, they are further converted via several pathways to be metabolised immediately to: generate energy via the glycolytic pathway; be stored as glycogen via glycogenesis; or converted to fat via *de novo* lipogenesis as depicted in *Figure 1.1.* Glucose can enter the same pathways, but unlike fructose and galactose that require liver specific enzymes for their initial conversion, glucose is able to undergo this metabolism in a large variety of cell types. Core metabolic processes involving carbohydrates are summarised in *Table 1.1.*

Table 1.1: Metabolic Pathways of Carbohydrate Metabolism

Pathway	Description	Primary Tissues
Glycogenesis	Conversion of glucose to glycogen for storage	Liver, and skeletal and cardiac muscle, although most tissues store some glycogen
Glycogenolysis	Breakdown of glycogen into glucose for energy production or release into the blood stream (liver only for the latter)	Liver, and skeletal and cardiac muscle
Glycolysis	Oxidation of glucose	Most tissue types

Pathway	Description	Primary Tissues
Gluconeogenesis	Conversion of noncarbohydrate compounds to glucose	Liver
Hexose monophosphate shunt	Production of 5-carbon monosaccharides and NADPH	Most tissue types
Tricarboxylic acid cycle	Oxidation of pyruvate and acetyl CoA	Most tissue types
De novo lipogenesis	Conversion of glucose metabolites to fatty acids	Liver and adipose tissue

Derived from Gropper *et al* (2008) [18].

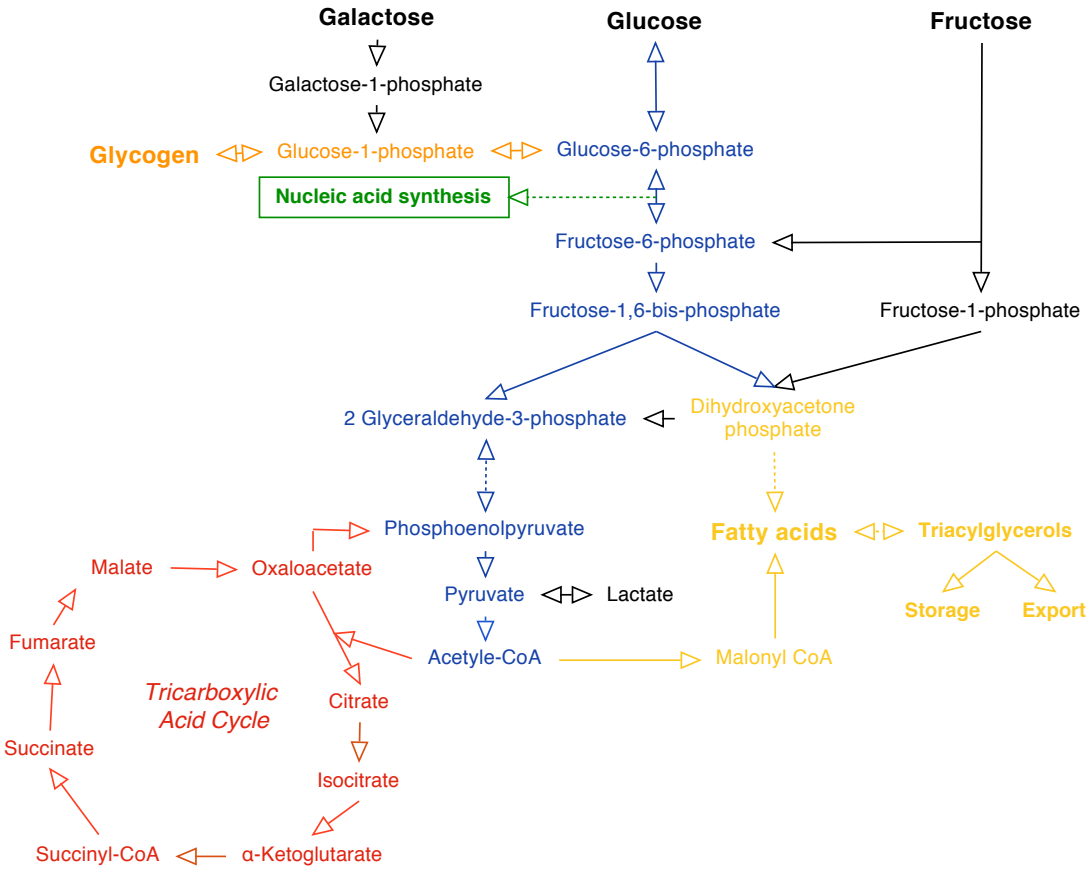


Figure 1.1: Hepatic carbohydrate metabolism showing the potential fate of fructose, galactose and glucose via the pathways of: glycogenesis in **orange**; glycolysis in **blue** into the tricarboxylic acid cycles in **red**; *de novo* lipogenesis in **yellow**; or the hexosmonophosphate shunt in **green**. Solid lines indicate direct conversion whereas interrupted lines indicate that one or more intermediate steps have been left out of the diagram. Sources: [16,18]

1.2.2.1 The Liver in Carbohydrate Metabolism

The liver, situated in the upper right of the abdomen and largest of the internal organs accounting for roughly 2-4% of body weight [35], is central to nutrient metabolism, including that of carbohydrates [18]. The liver is the second largest, after skeletal muscle, storage site for glucose, in the form of glycogen via glycogenolysis [18]. Following a mixed meal in a sedentary state, liver glycogen rises steadily for approximately four hours before reaching a plateau and then declines after six hours [36]. In contrast, hepatic glucose production declines sharply after the meal with baseline output not being paralleled until about six hours post consumption. The liver also represents the major site for *de novo* lipogenesis [37], which rises from ~5% after a 12 hour overnight fast to 23% (range:10-38%) after mixed meal and further after a second meal [38].

In the fasted state, hepatic net glycogenolysis and gluconeogenesis dominate to allow the export of glucose into the blood stream for the maintenance of blood glucose [18]. If fasting continues to the point where stored glycogen becomes minimal, usually after a few days, hepatic metabolism changes to channel more acetyl-CoA, predominantly from β-oxidation of fatty acids, towards the production of 3-hydroxy-3-methylglutaryl-CoA, which is further converted via multiple steps to acetoacetate, β-hydroxybutyrate, and propanone in the process of ketogenesis [39]. These ‘ketone’ bodies are then exported to supply the brain and other tissues with an energy alternative to glucose and lactate [39-42].

1.2.2.2 Adipose Tissue in Carbohydrate Metabolism

Adipose tissue accounts for 10-20% of systemic glucose use [43], and absorbs circulating glucose via GLUT4, GLUT10, and GLUT 12 [31,44]. Absorbed glucose can undergo glycogenesis, although the capacity for glycogen storage is lower in adipose tissue than it is in the liver or muscle [45]. As in the liver, exogenous glucose or that liberated by glycogenolysis can undergo *de novo* lipogenesis and be stored as TAG. In the fasted state adipose tissue glycogen can undergo glycogenolysis for the liberation of glucose.

1.2.2.3 Muscle in Carbohydrate Metabolism

Skeletal muscle, due to its total mass, has the greatest capacity for glycogen storage and is therefore a key tissue in blood glucose control [18]. Absorption of glucose into skeletal muscle is believed to occur predominantly via GLUT4 [31,46]. However, the glucose transporters GLUT5, GLUT11, GLUT12, and SGLT3 are also expressed in adult human muscle [30,31,47,48]. Once absorbed, glucose can undergo glycogenesis or be used in energy production via glycolysis leading to aerobic energy production via the tricarboxylic acid cycle and the electron transport chain, or anaerobic metabolism leading to lactate production [18].

1.2.2 Lipid Metabolism

Digestion of dietary lipids is initiated by chewing and the release of lingual lipase by the serous glands, continues in the stomach via the action of gastric lipase and mechanical emulsification from peristaltic motion [49], and culminates in the small intestine where emulsions mix with bile and pancreatic secretions [50]. Mechanical and enzymatic digestion of triacylglycerols (TAG) results in monoacylglycerols, non-esterified fatty acids (NEFA), and glycerol [49]. Phospholipid hydrolysis by phospholipases, of pancreatic origin, yields free fatty acids and lysophosphotidylcholine [50]. Cholesterol, and phytosterol and phytostanol-ester hydrolysis, by cholesterol esterase, yields unesterified cholesterol, phytosterol, and stanol, respectively [50,51]. Facilitated by bile salts and bile acids, these hydrolysis products of ingested and endogenous lipids form mixed micelles, the contents of which are absorbed by enterocytes [50].

Once inside enterocytes, these lipids can be: incorporated into the structure of the cell; oxidised; stored; or repackaged as TAG into chylomicrons for export into the circulation [50,52]. Lipids with carbon chain lengths >10 and not used as a fuel or incorporated into cell wall cholesterol esters or phospholipids, are converted to TAG and exported in chylomicrons, and to a lesser extent very low density lipoproteins (VLDL) [49,50]. Medium chain fatty acids – 6-12 carbons – mostly pass directly into the portal circulation unmodified [18]. Lipids can be released into either the portal vein or the lymphatic system in response to factors such as the form of the lipid, e.g. free fatty acid vs. TAG, the combination of lipids, and fatty acid chain length [49]. Among published findings, the most

consistent are that NEFA and shorter chain TAG are preferentially transported by the portal vein, whereas TAG packaged in chylomicrons are first released into the lymphatic system prior to entering blood circulation [49].

Appearance of ingested TAG, mostly incorporated into chylomicrons, in the circulation post-meal occurs in two phases [52]: the first phase, culminating in the 'early peak' at 10-30 minutes after the onset of the meal, involves release of lipids consumed in an earlier meal; and the second phase has its peak 3-4 hours post meal with lipids coming predominately from that meal. Circulating TAG concentrations continue to rise throughout the day with repeated meal consumption as each meal not only contributes directly to the TAG pool but also induces the release of TAG stored after prior meals in what has become known as the *second meal effect.*

Exchange of apolipoproteins from chylomicrons with other circulating lipoproteins provides sufficient apoC-II to facilitate TAG hydrolysis by extracellular lipoprotein lipase in small blood vessels and capillary beds, predominantly of skeletal muscle and adipose tissue [52,53]. Hydrolysis yields free fatty acids and diacylglycerols for absorption by surrounding tissue [52,53]. The decreased chylomicron size results in migration of excess phospholipids to high-density lipoprotein cholesterols, and a shift in cholesterol ester from the core to the surface of the resulting chylomicron remnants [53]. Incomplete absorption of liberated lipid results in *spillover* of NEFA into the circulation [52]. Metabolism of ingested lipids is depicted in *Figure1.2.*

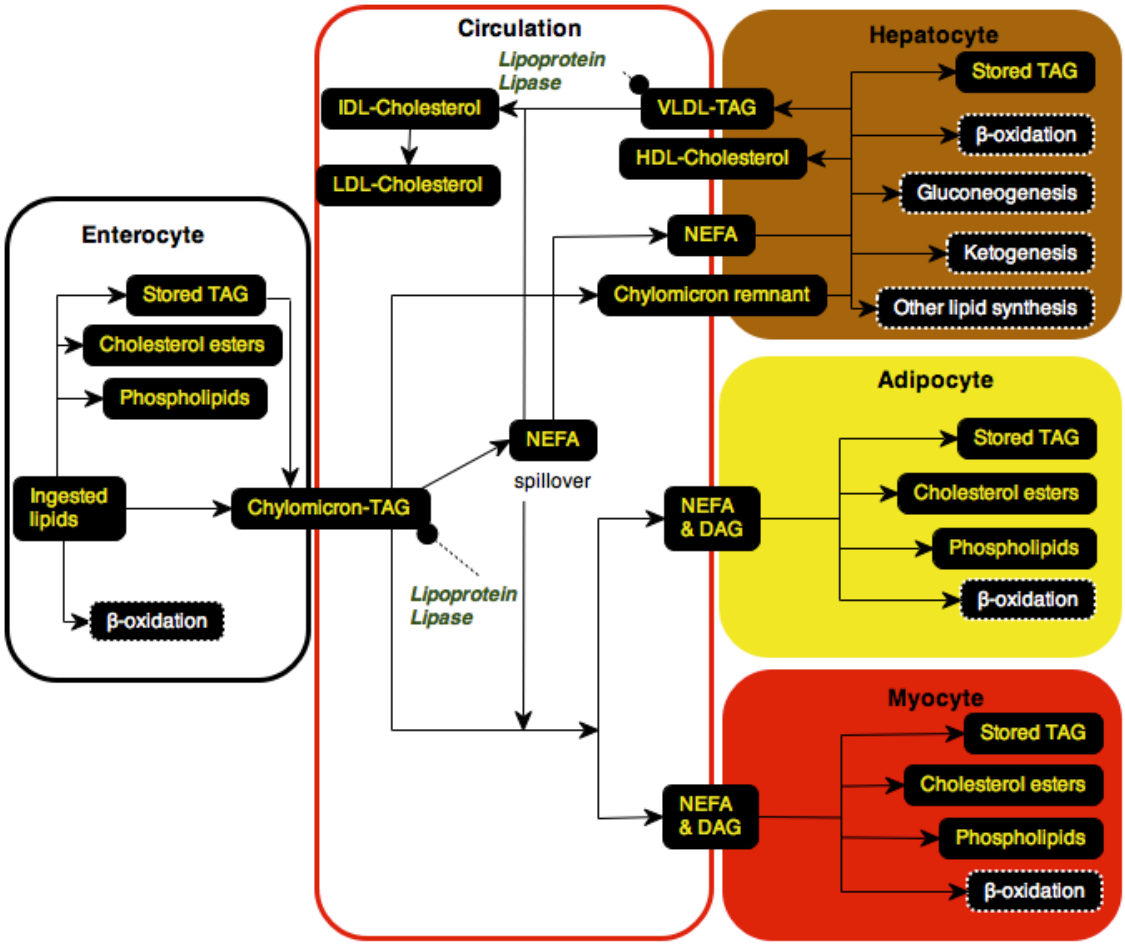


Figure 1.2: Following digestion of consumed lipids, the resulting glycerol, monoacylglycerols, and NEFA are absorbed by enterocytes. There they can undergo: oxidation for energy; reesterification to TAG for storage or incorporation into chylomicrons for export; or conversion into cholesterol esters or phospholipids. Exported chylomicrons are acted on by capillary bound lipoprotein lipase to release NEFA and diacylglycerol for absorption into surrounding tissue, predominantly adipose and muscle. TAG-depleted chylomicrons become chylomicron remnants that are absorbed by the liver along with circulating albumin bound NEFA from its incomplete absorption by other tissue. The liver exports some of the absorbed and reconstituted TAG in VLDL, which is also a target for lipoprotein lipase. TAG-depleted VLDL transiently becomes IDL before forming the cholesterol rich LDL particle after TAG is removed. This LDL-cholesterol is then available for absorption by tissues (not depicted). Diagram based on sources: [18,39,52].
Abbreviations: DAG, diacylglycerol; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; NEFA, non-esterified fatty acids; TAG, triacylglycerol; VLDL, very low density lipoproteins.

1.2.2.1 The Liver in Lipid Metabolism

Digestion of lipids is dependent on bile salts produced by the liver [18]. The liver first comes in direct contact with ingested lipids in the form of circulating NEFA released from chylomicrons by lipoprotein lipase in other tissues, and chylomicron remnants, which it absorbs via endocytosis [18,52]. Absorption of lipids is facilitated by membrane proteins including: caveolins; fatty acid translocase/cluster of differentiation 36 (FAT/CD36); fatty acid transport proteins (FATP); and plasma membrane-associated fatty acid-binding protein (FABP) [17].

Net uptake of meal TAG by the liver is 8-12% [52]. Absorbed lipid can be stored in TAG droplets, converted into phospholipids and cholesterol esters, metabolised for energy and/or intermediates mainly for gluconeogenesis or ketogenesis, or repackaged into very low density lipoproteins for export [54]. However, in the postprandial state, assuming an adequate carbohydrate supply, the primary fuel source for the liver is glucose, thus postprandial metabolism favours storage or export rather than oxidation of fatty acids [52]. During the fasted state the main exogenous lipid supply comes from circulating NEFA [17]. Stored TAG is broken down to supply substrate for gluconeogenesis, ketogenesis, and mitochondrial β-oxidation as described above [18,39].

1.2.2.2 Adipose Tissue in Lipid Metabolism

Adipose tissue absorbs lipid liberated from chylomicrons by lipoprotein lipase and predominantly stores these in TAG droplets [18,52]. In the fasted state there is net export of NEFA from adipose tissue [45]. The majority of this adipose-derived circulating NEFA appears to come from abdominal subcutaneous adipose tissue [55]

1.2.2.3 Muscle in Lipid Metabolism

As noted above, TAG bound in chylomicrons is liberated by the hydrolytic action of the capillary membrane bound lipoprotein lipases and the resulting NEFA and DAG absorbed by nearby tissue. Absorption of lipid into the muscle proceeds by both passive diffusion and protein mediated transport via: FAT/CD36; FABP; and FATP 1 and 4 [56]. Once absorbed, the lipids can undergo much the same processes as described for adipose tissue, except they do not appear to released back into the circulation [18].

1.2.3 Hormonal Control of Hepatic and Systemic Metabolism

The pancreatic hormones insulin, produced by the β-cells, and glucagon, produced by the α-cells, are the main acute regulators of both carbohydrate and lipid metabolism [16]. Insulin secretion increases to parallel the elevation of blood glucose in the postprandial period following carbohydrate consumption [57-59].

Elevated insulin promotes hepatic glycogenesis, down regulates gluconeogenesis, and decreases *de novo* lipogenesis [16,60]. In skeletal muscle, glucose uptake is increased via insulin mediated translocation of GLUT4 and GLUT12 to the cell wall [61]; glycogenesis also increases [16]. And in white adipose tissue, elevated insulin decreases release of NEFA into the blood stream and promotes both glycogenesis and *de novo* lipogenesis [45]. Insulin thus promotes a shift in metabolism to favour glucose as the dominant fuel source for cellular metabolism.

During states in which blood glucose concentration decreases within the normal range or below, such as in the fasted state or while exercising, more glucagon and less insulin are produced thus lowering insulin:glucagon ratio [16]. The increase in glucagon increases hepatic glycogenolysis, decreases glucose uptake, and results in a net export of glucose into the blood stream [62]. Over prolonged periods of hypoglycaemia, catecholamines augment the function of glucagon [62]. Reduced insulin concentration leads to increased gluconeogenesis, decreased glycogenesis and *de novo* lipogenesis in the liver [63], and increased release of NEFA and glycerol from the breakdown of stored TAG in adipose tissue [16]. The effect of decreased circulating insulin on skeletal muscle depends on whether the muscles are resting or engaged in forceful contraction, such as during exercise or physical labour. During exercise glucose uptake remains elevated in contracting muscle independent of circulating insulin [64]. At rest, reduced insulin concentration leads to reduced absorption of circulating glucose and increased intramuscular glycogenolysis [16].

1.2.4 Common Modifiers of Postprandial Macronutrient Metabolism

In healthy humans free from metabolic disease and not taking medication that might affect metabolism, the key modifiers of postprandial metabolism are: composition of the meal in question [52,65,66], and the preceding meal [36,67,68]; physical activity prior to or shortly after the meal, and time of day [69]. Meal composition determines insulin and glucagon response in that meals containing digestible carbohydrates and/or proteins lead to increased insulin release [18,65,70], and in the case of protein, a variable glucagon response [70]. No such change is observed with meals exclusively made of lipids [18].

The rate of absorption and appearance of dietary glucose in the blood is highly variable and dependent on both dietary and host factors [71]. Nonetheless, multiple approaches to quantifying the glucose response to different carbohydrate sources exist [65]: the glycaemic index (GI) compares the area under the blood glucose curve for a food or meal containing a standard carbohydrate (50 g) dose with the response for an equal amount of glucose in solution; the glycemic load is the product of GI multiplied by the carbohydrate content of the food or meal; and glycemic equivalence is established by measuring the response to food or meals of non-standard carbohydrate content.

Several factors have been shown to modify glycaemic response to similar foods including the extent of chewing [72], the presence of fat and/or protein [70,73,74], the specific type of protein [75], physical and chemical attributes of foods including processing such as freezing and toasting [76], and cooking method [77]. Being in a postprandial or postabsorbtive as opposed to a fasted state has also been shown to reduce the glycaemic response to a meal [36]. Further, a prior carbohydrate containing meal increases the amount of glucose directed toward liver glycogen synthesis [78]. This second meal response, noted for lipids above (section 1.2.2.), occurs even if meals are separated by an overnight fast [67].

Just as postprandial response to one meal is influenced by prior meals, so prior meal composition can influence metabolism in exercise following a meal. For example, meals with a lower glycaemic index are more likely to promote higher fat oxidation from both circulating NEFA and intramyocellular lipid stores, whereas meals with a higher glycaemic index favour carbohydrate oxidation and lower availability of NEFA as a fuel source [67,79-81]. The corollary is that physical activity, including exercise, also modifies postprandial metabolism. Simply breaking up two hours of sedentary time with two minutes of light-to-moderate intensity walking every 20 minutes reduced postprandial glucose and insulin response [25]. Higher intensity activities preceding or shortly after a meal have also been shown to: increase lipid oxidation [27]; reduce plasma lipid response [28,82-85]; improve lipoprotein profile [86,87]; reduce plasma insulin and/or glucose response [28,83,88,89]; and suppress *de novo* lipogenesis [90]. However, not all findings are consistent with some reports indicating that an acute bout of

prolonged resistance exercise can elevate postprandial TAG area under the curve [91], and that post meal lower-intensity exercise is ineffective at modifying postprandial metabolism whereas the same exercise prior to meals reduces glucose response [92].

Exercise transiently increases insulin independent skeletal muscle glucose absorption [64], and hepatic glycogenolysis in an exercise intensity dependent manner [63]. As hepatic glycogen stores diminish, gluconeogenesis increases [63]. Forceful muscle contraction also increases myocellular insulin sensitivity and hence insulin mediated glucose absorption mediated by increased expression of GLUT4 and GLUT12 [61]. The elevation in GLUT4 mRNA production lasts less than 24 hours, but there is a cumulative effect of daily exercise in terms of GLUT4 protein expression [93]. Increased insulin sensitivity following exercise reduces postprandial *de novo* lipogenesis [26]. Exercise also elevates the activity of lipoprotein lipase [94], and muscle contraction drives the relocation of the fatty acid transport protein FAT/CD36 to the plasma membrane of myocytes, thereby contributing to enhanced uptake of circulating lipids [95].

1.3 Metabolism in Non-Alcoholic Fatty Liver Disease

Obesity, insulin resistance, and the presence of hepatic steatosis all contribute to altered fasting and postprandial metabolism both systemically and in specific tissues, particularly the liver, adipose tissue, muscle, and the pancreas [96]. In NAFLD, the most fundamental change is an imbalance between hepatic lipid uptake and *de novo* lipid production relative to overall export leading to net hepatic fat accumulation [17].

In obese volunteers with hypertriglyceridaemia and NAFLD circulating NEFA, hepatic *de novo* lipogenesis, and dietary lipids accounted for 59±10%, 26±7%, and 15±7% of labelled IHTAG after four days of a labelled mixed diet, respectively [97]. The same study also showed chronically elevated *de novo* lipogenesis without the diurnal and fasted vs. postprandial variation found in healthy volunteers [38,97]. This elevation of hepatic *de novo* lipogenesis had been previously demonstrated in an insulin resistant NAFLD cohort relative to healthy controls [98], and also obese and insulin resistant cohorts of unknown NAFLD

status [99-101]. Elevated *de novo* lipogenesis is accompanied by a reduction in hepatic and muscle glycogen synthesis in conditions of insulin resistance [78,102-104].

In addition to increased *de novo* lipogenesis, volunteers with an IHTAG over 6% also exhibited a 25% higher rate of gluconeogenesis than those with IHTAG under 6% [105]. The degree of change in gluconeogenesis was greater when NAFLD was accompanied by overweight/obesity [106].These findings in NAFLD cohorts are consistent with earlier observations that gluconeogenesis is elevated in obesity and linked to increased postabsorptive protein breakdown [107].

Adipose tissue storage of fat was reduced after meals in abdominally obese men relative to lean controls [108]. In diet controlled type 2 diabetes, postprandial absorption of fatty acids into skeletal muscle and hepatic TAG pools is greater and more rapid following a mixed meal relative to healthy controls [109]. Hepatic lipoprotein lipase activity is elevated in NAFLD [110], and was 4.5 times higher in morbidly obese type 2 diabetics prior to vs. post massive weight loss through bariatric surgery [111]. The transmembrane lipid transporter FAT/CD36 protein and/or gene expression is reduced in the adipose tissue of those with NAFLD, while it is increased in their hepatic and muscle tissues [112,113]. Thus availability of lipid to the liver appears to be matched by increased absorption.

Plasma concentrations of ß-hydroxybutyrate indicate hepatic fatty acid oxidation similar or up to two-fold greater in NAFLD than in healthy controls [105,114-116]. Also, VLDL-TAG secretion increases linearly with liver fat concentration, but plateaus at 10% [117]. Further, synthesis of apoB-100, a protein component of VLDL, was reduced in NASH relative to obese and lean controls suggesting a reduced capacity for VLDL-TAG synthesis and thus export [118]. Nonetheless, although comparable in the fasting state, plasma VLDL and TAG increased more in simple steatosis and NASH cohorts than in healthy controls following a fat tolerance test [119]. These findings suggest that in NAFLD the total VLDL-TAG clearance rate is reduced, adipose tissue clearance is particularly impaired, but muscle and liver lipid absorption are increased.

Patients with biopsy-confirmed NASH had greater adipose and hepatic insulin resistance, and poorer β-cell function than patients with simple steatosis or non-NAFLD controls matched for abdominal adiposity and symptoms of the metabolic syndrome [119]. The difference in β-cell function remained even after excluding patients with impaired glucose tolerance. Further, elevated IHTAG is associated with reduced insulin clearance, and hepatic insulin resistance [120]. Elevated intramuscular lipid is similarly associated with muscle insulin resistance [121], and liver fat concentration was found to be closely associated with intramuscular fat concentration when assessed at baseline, and following a weight reduction intervention [122].

Collectively, these observations from NAFLD and closely related cohorts indicate that NAFLD at various stages is associated with as yet not fully characterised changes in postprandial macronutrient metabolism, and to a lesser extent fasting metabolism. These changes result in, and derive from, a disproportionately high ectopic lipid storage best characterised in skeletal muscle and the liver, which in turn contribute to central and peripheral insulin resistance.

1.4 Definition and Assessment of Non-Alcoholic Fatty Liver Disease

NAFLD refers to a spectrum of conditions ranging from simple hepatic steatosis, non-alcoholic steatohepatitis, to fibrosis, and cirrhosis [1]. Its early stage is defined by excess fat in the liver in the absence of excessive alcohol intake, other liver pathologies such as viral and autoimmune disease hepatitis, and pharmacological causes [13,14], and is therefore a diagnosis by exclusion. However, no single objective and quantitative definition for NAFLD exists as multiple techniques are used to assess liver fat; some of which are semi-quantitative in nature (see below). If analysed by biopsy, 5% of hepatocytes containing visible fat droplets is considered the lowest end of steatosis [123]. In the case of assessment by proton magnetic resonance spectroscopy (^{1}H-MRS) the threshold for excess liver fat has been variously established at 5.6% and 3% [124,125]. In both cases based on the 95th centile of an apparently healthy non-insulin resistant sample. The definition of what constitutes excessive alcohol

intake and the methods used to assess intake also vary considerably across studies of NALFD [15].

The primary characteristic of the early stages of NAFLD, hepatic steatosis can be identified by ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), ^{1}H-MRS, and histological examination of biopsy samples [126]. CT, MRI, and ^{1}H-MRS are quantitative methods of analysis, whereas ultrasound and histological analysis are semiquantitative [126].

1.4.1 Biopsy

Histological examination, by light microscopy, of biopsy samples can assess the presence of necroinflammation and fibrosis, and can differentiate between macro- and micro-vesicular steatosis as shown in *Figure 1.3*; it remains the reference standard for the grading and staging of NAFLD [13,14], but caries some risk due to its invasive nature [127,128]. To date, no imaging or other noninvasive techniques exist that can fully replace histological examination in terms of grading and staging NAFLD [129,130]. However, biopsy is subject to sampling error [131-133], and inter-rater differences in scoring/grading are recognised limitations [123,133,134]. The potential for sampling error exists due to tissue heterogeneity between lobes [131], and even closely adjacent areas [135,136]. Biopsy length and fragmentation also influence diagnosis [137,138]. Further, the scoring/grading systems have evolved over time and not all are suitable for scoring the full spectrum of NAFLD [123].

Histological examination of liver biopsies has been used to validate imaging techniques [139-142]. The key caveat to this approach is that it involves comparing a semi-quantitative subjectively scored technique with more quantitative and objective techniques. More objective, e.g. computer-assisted, quantitative methods of histological and histochemical biopsy assessment have been investigated and advocated, but not routinely adopted in clinical care or research [134,139,143,144].

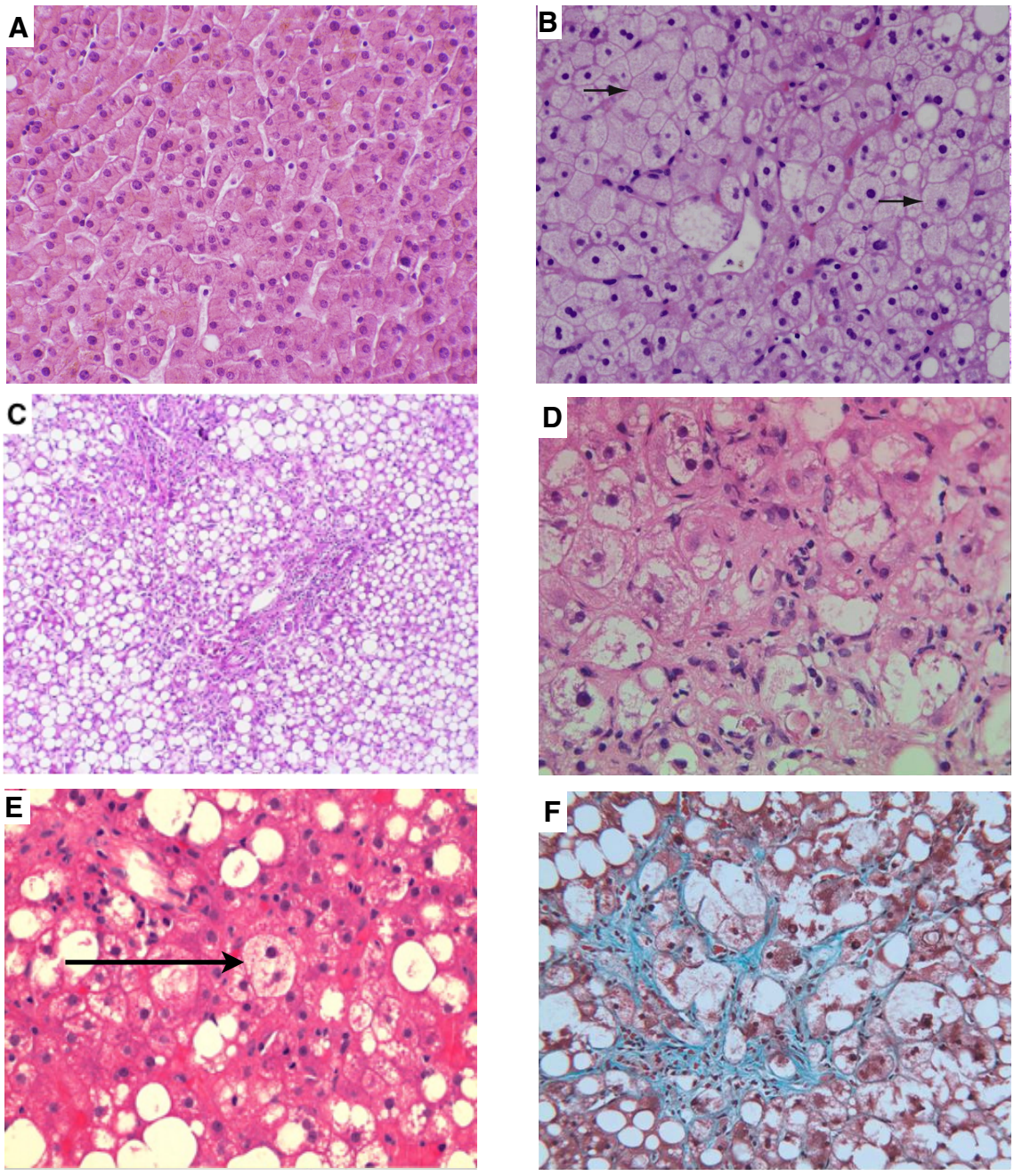


Figure 1.3: Liver histology under light microscopy: A.normal liver; B. microvesicular steatosis shown by arrows; C. notable macrovesicular steatosis; D. inflammation with steatosis; E. fatty liver ballooning shown by arrow; F. steatosis with fibrosis (stained blue).

1.4.2 Magnetic Resonance Spectroscopy and Imaging

Magnetic resonance scanners provide images or chemical spectra by using a very large permanent magnetic field in conjunction with other temporary applied electromagnetic radiation [145]. The magnetic field causes the angular momentum of the atomic nuclei, known as *spins*, to be quantized to two energy states by aligning the spins to face either the same direction as the main field – *up* – or directly against it – *down*. A radio frequency pulse with a frequency specific to the nucleus of interest – the *resonant frequency* – is applied to alter the distribution of spins in the up and down states. Cessation of the pulse allows these nuclei to return to their original state resulting in the release of a measurable radio frequency pulse at the resonant frequency of the nuclei concerned. The timing and frequency of this emitted pulse is modified by the chemical environment of the nuclei, and this information is used, either with spatial encoding to construct an image, or to generate spectra.

Liver fat can be measured by focusing on the spectra created by the single proton nuclei of hydrogen atoms that are abundant in both water and fat [146]. As illustrated in *Figure 1.4*, the nuclei of hydrogen in water resonate at a slightly different frequency, 435 Hz difference at 3 T, to those in fat. The frequency thus identifies the chemical responsible for each peak, i.e. water or fat, and the area of the peak gives the quantity of nuclei detected. The volume of tissue to which the spectra corresponds is the *volume element* or *VOXEL*, whose size can be varied but is usually several cubic centimetres [147].

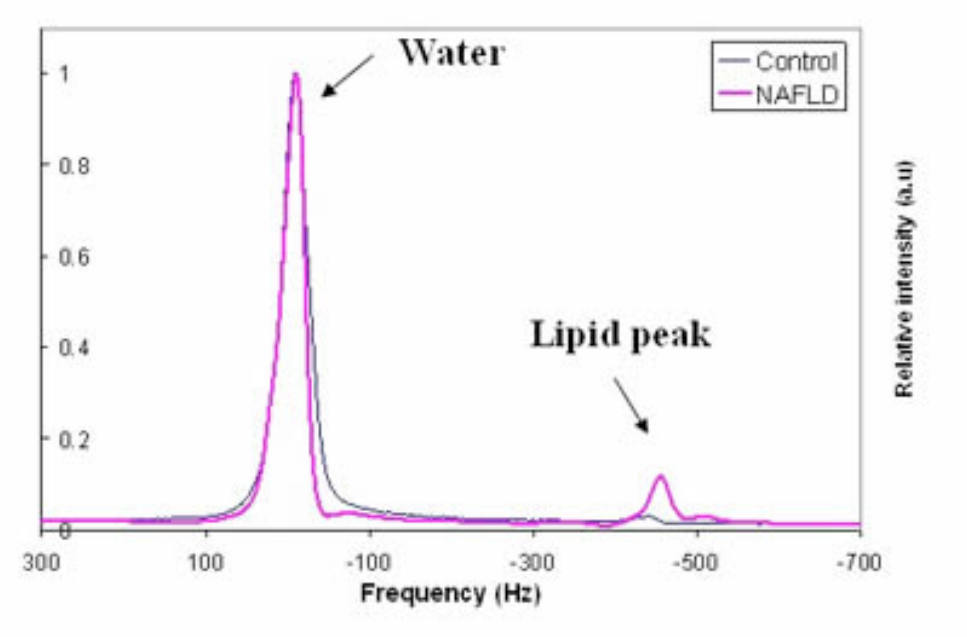


Figure 1.4: Water and lipid spectrum from liver ^{1}H-MRS in a control volunteer and one with NAFLD

An alternative to spectroscopy is 3-Point Dixon MRI [148]. For this, three gradient echo scans of several liver sections going from superior to inferior are acquired. The three adjacent scans comprise of one that is out-of-phase (the water and fat signal cancel), one that is in-phase (the water and fat signals are additive), and an out-of-phase echo [149]. The resulting data are processed to yield separate fat and water images, and software used to determine fat percentage derived from the contrast of images. Fat content is reflected in how dark or light the slice is with more fat resulting in lighter slices (see *Figure 1.5*).

^{1}H-MRS provides a non-invasive fully quantitative measure of liver fat, unlike biopsy, and it has superior accuracy and sensitivity to CT and ultrasound [140]. The technique has also been validated against biopsies [141,150]. The key limitation of ^{1}H-MRS relative to biopsy is its present inability to reliably detect other components of NAFLD such as inflammation and fibrosis [151]. The other notable limitation of ^{1}H-MRS is that it examines only a portion of the liver, the designated VOXEL. However, at several cubic centimetres this area is several-fold larger than that obtainable by biopsy.

MRI makes it feasible to examine a larger portion of the liver, and makes it easier to assess the homogeneity of liver fat distribution [148]. However, imaging methods have been less extensively validated than spectroscopy [147].

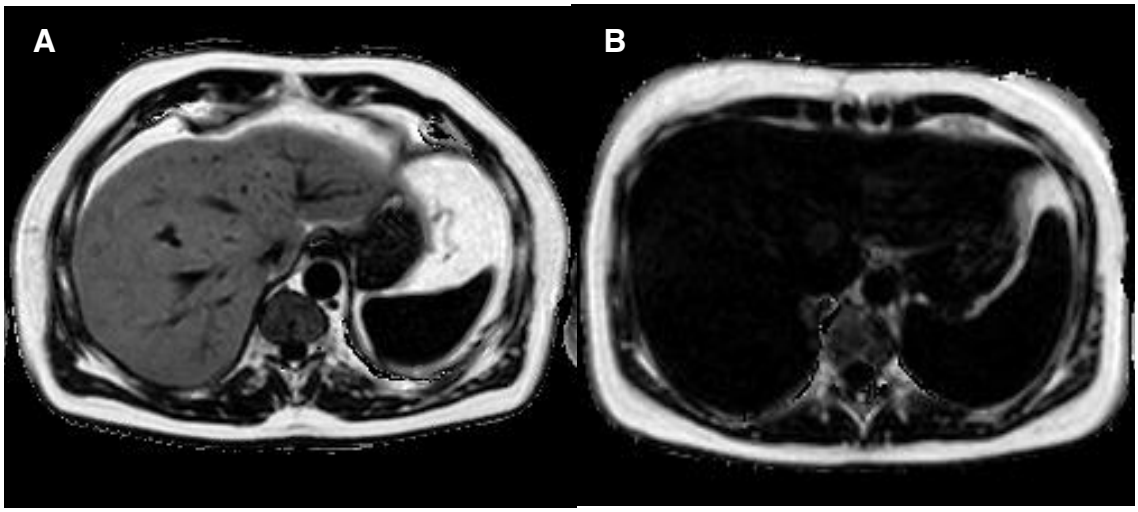


Figure 1.5. shows two 'fat only' magnetic resonance images of livers. Image A is from a volunteer with hepatic steatosis – approximately 32% liver fat – as indicated by the relative brightness of the liver. Image B is from a control volunteer without hepatic steatosis – approximately 3% liver fat – as indicated by the relative darkness of the liver.

1.4.3 Computed Tomography

CT provides a semi-quantitative method for the evaluation of IHTAG based on the change in image intensity, measured in Hounsfield units, between the liver and either the spleen, which stores no fat, or an external lipid standard [126]. An increase in liver:spleen ratio or liver density is indicative of reduced IHTAG.

1.4.4 Ultrasound

Ultrasound is the most readily available and inexpensive imaging technique for assessing degree of steatosis. It provides a semi-quantitative assessment of liver fat based on diffuse increases in image echogenicity with reported sensitivities and specificities varying between 60-94% and 66-95%, respectively [126]. Sensitivity is improved when liver fat exceeds 30% [152]. However, sensitivity is reduced in the morbidly obese in whom the prevalence of NAFLD is particularly high [153]. Inter- and intra-observer variability for the assessment of the presence of steatosis has been reported as 72% and 76%, respectively, with an observer agreement of severity of 55-68% [154].

1.4.5 Liver Enzymes

Liver enzymes, specifically alanine aminotransferase (ALT), aspartate aminotransferase (AST), and/or γ-glutamyl transferase (GGT), are sometimes used in the diagnosis and/or tracking of NAFLD where other common causes for hepatic steatosis have been ruled out [15]. However, liver enzymes are markers of liver injury [155], and are non-specific for the presence, degree, or progression of NAFLD.

In a cohort of 708 individuals with elevated intrahepatic lipid (>5.6% assessed by ^{1}H-MRS) 79% had normal ALT [156]. Larger ultrasound assessed cohorts show similar discrepancies between the presence of steatosis and transaminase concentrations [157,158]. Normal ALT and AST concentrations have also been reported in the presence of histological evidence of steatosis, fibrosis, and cirrhosis [159-162]. Even when the conservative serum ALT cutoff of19 IU was employed, sensitivity and specificity for NAFLD remained poor at 74% and 42%, respectively [163]. Further, some clinical cohorts predominantly display raised GGT, not ALT, concentrations in the presence of NAFLD [164,165].

As well as being a poor static measure, 3-monthly ALT, AST, and GGT measurements in 73 untreated NAFLD patients with repeat biopsy data, at baseline and two years, showed a mean downward trend in all three enzymes despite no change in body weight [166]. There was no significant correlation between liver enzyme and histological profile change.

Despite the recognised limitations of individual biomarkers, multiple biomarkers in combination with other data such as diabetes status have been successfully used to develop composite scores that predict risk of more advanced liver disease, for example the NAFLD fibrosis score [167]. The use of ALT:AST ratio is also predictive of fibrosis [168]. Further, recent work suggests that a specific form of GGT, *big* GGT, may be more predictive of and specific to NAFLD [169].

1.4.6 Stability of Liver Lipid Concentration and Time Course of Changes

No work describing either circadian or serial day-to-day variation in liver lipid concentration under free living or specific intervention conditions has been published. The temporal variation in two key contributors to liver lipid, circulating NEFA and *de novo* lipogenesis, suggest IHTAG could vary accordingly. Day-to-day serum NEFA concentrations have an intra-individual coefficient of variation (CV) of 45%; although there appears to be a trend over time as indicated by measures greater than two weeks apart showing a CV of 24% [55]. Further, *de novo* lipogenesis is elevated during the follicular phase of menstruation [99]; potentially leading to differences in IHTAG by menstrual phase.

Nonetheless, ^{1}H-MRS quantified IHTAG after an overnight fast appeared stable over four weeks with CVs of 4.1% and 9.5% in volunteers with NAFLD (n = 8) and a mixed group of NALFD and non-NAFLD volunteers (n = 24), respectively [170]. The within 4-hour CV was 4.5% in a mixed NAFLD and non-NAFLD group with both scans performed in the fasted state (n = 12). Further, no significant difference in IHTAG, assessed by ^{1}H-MRS, was found when comparing measurement after a 10-hour overnight fast and 4-hours post high-fat – 50 g of fat – breakfast on the same day [124]. The study was tightly controlled with an inpatient protocol, but there were only eight participants of whom only one had an IHTAG above 5%. The meal was almost exclusively fat, whereas there is reason to believe carbohydrates may have a much stronger acute effect on IHTAG.

Comparison of two hypocaloric diets, both1000 kcal below estimated requirement, in NAFLD cohorts, showed a much greater reduction in IHTAG after 48 hours when carbohydrate was restricted to 20% of energy than when fat was restricted to 10% of energy: IHTAG decreased from 12.4% to 8.7% and. 11.2 % to 10.2%, respectively [171]. One week on a 600 kcal/day diet in an overweight cohort with type 2 diabetes, resulted in a similar clear and rapid reduction in IHTAG from 12.8% to 9.0%, [172]. Two weeks of an 8% carbohydrate diet led to greater reductions than a 50% carbohydrate diet – IHTAG 22% to 10% vs. 19% to 14%, respectively [173]. Tracer work indicating that *de novo*

lipogenesis accounts for a greater proportion of IHTAG than dietary lipid would seem to offer an explanation for these findings (see *Section 1.3*) [97].

In healthy non-NAFLD volunteers caloric restriction in the form of fasting has shown variable effects. Twelve hours of fasting resulted in no change in liver fat [174]; Twenty four hours of fasting reduced liver fat by 34% (only relative decrease reported)[175], Conversely, 36 and 48 hours of fasting lead to increased IHTAG in men [174,176]. Whereas 48 hours of fasting left liver fat concentration unchanged in women; who instead showed increased intramuscular lipid accumulation [176].

Although none of these studies set out to systematically evaluate the time course of IHTAG changes under the conditions studied, they show that IHTAG concentration can respond rapidly to changing energy and macronutrient intake, and that responses to energy restriction may differ substantially between those with NAFLD and those with low IHTAG.

1.5 Symptoms

The most commonly reported symptoms of NAFLD and NASH are pain near the liver, general fatigue, and/or a noticeably enlarged liver, but the majority of patients report no symptoms [177,178]. Studies have also observed a link with fatigue and daytime sleepiness, and identified overall reduced quality of life [179,180], autonomic dysfunction [180], and reduced exercise capacity [181].

In a cohort of 156 with histologically-confirmed NAFLD assessed by a validated fatigue specific questionnaire, fatigue was considerably higher than in a control cohort – 51±38 vs. 8±12 [179]. The same cohort also took fewer daily steps than controls, indicating less physical activity, and reported worse overall quality of life. However, NAFLD severity was not correlated with fatigue severity. Self-reported fatigue was found to correlate with autonomic dysfunction in a cohort of 34 non-diabetic, non-cirrhotic patients with histologically confirmed NAFLD [180]. Specifically, questionnaire assessed fatigue correlated positively with orthostatic symptoms, and inversely with 24-hour ambulatory blood pressure.

The absence of self-reported symptoms in the clinical setting but presence on specific investigation suggests that patients may simply not be fully aware that

their physical state is suboptimal, or that they attribute this to non-medical factors. It does however suggest that improvements in autonomic function and reduced fatigue would be perceived as tangible benefits to therapy to motivate adherence.

1.6 International Prevalence of Fatty Liver

Good data on national prevalences of non-alcoholic or indeed alcoholic fatty liver disease are scarce due to a lack of population representative sampling and frequent reliance on less sensitive and/or specific methods for measuring steatosis [5]. Even when hepatic steatosis is clearly established, ruling out the contribution of alcohol intake is complicated by the difficulty in accurately measuring alcohol intake as well as inconsistent definitions of *moderate* intake [15,182].

A recent systematic review of published adult cohorts indicated an international prevalence of NAFLD of 9-33% [5]. Estimates in children and adolescents of approximately 3%, are based on a much smaller dataset than prevalence in adults [6]. *Figure 1.6* shows regional and demographic distributions of fatty liver prevalence using either ultrasound or ^{1}H-MRS. The summarised data illustrates regional differences, as well as an influence of ethnicity. Texas-based hispanics had higher prevalence than caucasians from the same region, and African-Americans had the lowest prevalence [156,183]. NAFLD prevalence also increases with increasing obesity and the presence of type 2 diabetes [5]

Data on the prevalence of NASH are even more limited due to the need for histological assessment to confirm the diagnosis. One study of a volunteer cohort (n = 328) recruited from a military primary care facility in Texas evaluated steatosis by ultrasound and followed up those with the appearance of NAFLD with biopsy assessment [183]. The prevalence of NASH in this cohort was 12%.

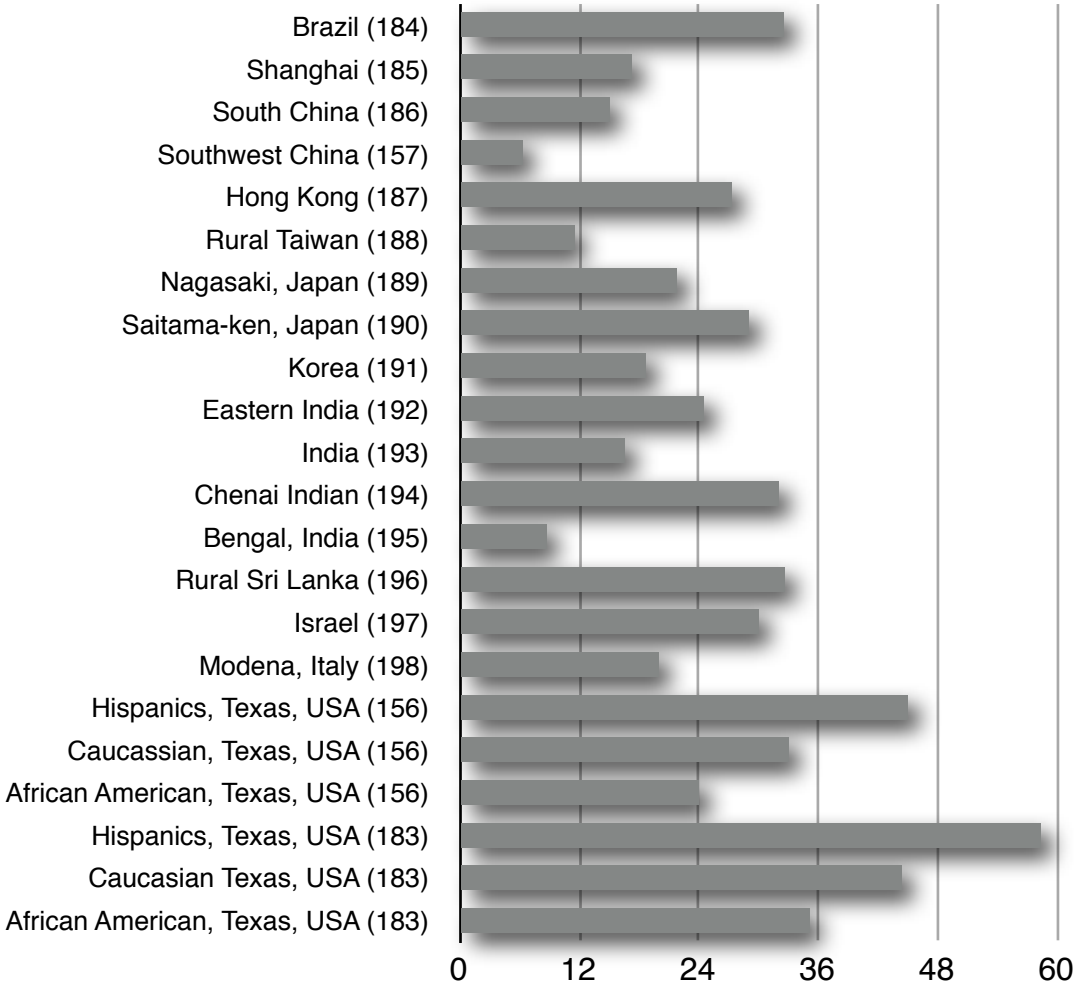


Figure 1.6. International Adult Percentage Prevalence of Non-Alcoholic Fatty Liver Disease
Source references: [156,157,183-198]

1.7 Risk Factors

The prevalence data above already defines some key risk factors for NAFLD, notably: overweight/obesity, type 2 diabetes, age, and hispanic ethnicity. These are all easy to assess. As noted in *Section 1.4*, NAFLD is a diagnosis by exclusion, the most commonly excluded contributors to hepatic steatosis being: excessive alcohol consumption; viral and autoimmune hepatitis, human immunodeficiency virus; some medications; haemachromatosis; certain gastrointestinal surgical procedures being most commonly excluded by objective measures [13,14]. Other contributors to hepatic steatosis are not routinely excluded and whether these fall under the umbrella of NAFLD is open to debate [199]. *Table 1.2* outlines both common and rare factors associated with hepatic steatosis.

Table 1.2: Factors Associated with the Development of Hepatic Steatosis

Category	Substance/Condition
Toxic causes	Alcohol
	Toxins: thinners, solvents, rapeseed cooking oil, dimethylflormamide, petrochemicals, pesticides
	Cocaine
	Pharmaceuticals: nifedipine, diltiazem, diethylaminoethoxyhexestol, tamoxifen, oestrogens, corticosteroids, methotrexate, perhexiline, amiodarone, highly active antiretroviral therapy
Nutrition	Total parenteral nutrition
	Starvation related: cachexia, rapid weight loss, anorexia nervosa, marasmus, kwashiorkor
Surgical procedures	Bypass surgery: bariatric surgery, Scopinaro procedure
	Whipple procedure (removal of part of the pancreas)
	Blind loop with bacterial overgrowth
Infectious agents	Hepatitis C, B, and D viruses
Inborn errors of metabolism	Amino acid disorders: heptorenal tyrosinaemia type I, homocystinuria, type II citrullinemia, neonatal intrahepatic cholestasis
	Bile acid synthesis defects
	Carbohydrate disorders: Galactosaemia, hereditary fructose intolerance, glycogen storage diseases (type I, III, IV, VI, Fanconi-Bickel)
	Channelopathy: Cystic fibrosis
	Disorders of glycosylation
	Lipid & fatty acid disorders: hypobetalipoproteinaemias, α- & β-oxidation defects, Dorfman-Chanarin syndrome
	Wilson disease
	Mitochondrial disorders: Alpers syndrome, mitochondrial DNA depletion syndrome
	Organic acidoses: propionic acidaemia, methylmalonic acidaemia, methylcrotonyl acidaemia, methylglutaconic acidaemia
	Peroxisomal disorders: adrenoleucodystrophy, Zellweger syndrome
	Protein folding disorders: α1-antitrypsin deficiency
	RNA processing disorders: Shwachman-Diamond syndrome
Other disorders	Polycystic ovary syndrome

Category	Substance/Condition
	Alstrom syndrome
	Bardet-Biedl syndrome
	Prader-Willi syndrome
	Lipodystrophy syndromes
	Insulin receptor & post-binding defects
	Thyroid disorders
	Hypothalamo-pituitary disorders
	21-α-hydroxylase deficiency
	Celiac disease
	Inflammatory bowel disease
	Hepatic Weber-Christian disease

Adapted from reference Cassiman and Jaeken [199]

Many of the factors outlined in *Table 1.2* that are not already specifically excluded from the spectrum of NAFLD, are rare, or at least rare in economically more developed countries, and it is therefore reasonable to assume that the majority of the NAFLD disease burden can be attributed to more prevalent risk factors explored below. Nonetheless, the heterogeneity within the aetiology of NAFLD may in part explain any heterogeneity in treatment response.

1.7.1 Overweight and Obesity

The prevalence of NAFLD increases with increasing obesity as defined by body mass index (BMI) and increased adiposity, particularly abdominal visceral adiposity [5]. Understandably, prevalence also increases with waist circumference as it is closely associated with visceral adiposity [200].

1.7.2 Gender

Surveys of disease prevalence conflict with respect to whether gender is a risk factor and if so whether males or females are at greater risk [5].

1.7.3 Age

The prevalence and severity of NAFLD increase with age [5,183,187,201]. The association between age and prevalence of NAFLD has been shown in several populations: Northeast England [201]; Hong Kong [187]; Texas [183]; India [193]; China [157]; and Korea [191]. Several cohorts also suggest that age increases the risk of NASH and fibrosis [202-205]. The absence of good quality data on physical activity and measures of cardiorespiratory fitness in these cohorts is noteworthy, as these are potential modifiers of the association between liver health and age (see *Section 1.7.5* below).

Interestingly, in an octogenarian cohort no association between the high prevalence of fatty liver and metabolic disease was found [206]; suggesting the disease phenotype is different for the longer-lived.

1.7.4 Diet

The close association between adiposity and NAFLD, and between excess energy intake and adiposity, suggests a clear link between energy intake and NAFLD. The rapid response of liver fat to energy restriction described in *Section 1.3.*7 and *Chapter 2* illustrate the close link between liver fat and energy balance. Patients with NAFLD and NASH have been reported as having higher energy intakes than healthy controls in some [207], but not all reports [208]. Liver fat has also been associated with soft drink consumption [209,210], overall fructose consumption [211], and overall carbohydrate consumption [212], and inversely associated with very long chain n-3 polyunsaturated fatty acid intake [213], and low liver and circulating n-3 and n-6 polyunsaturated fatty acids [208]. Experimental data also suggest a deleterious effect of high saturated fat consumption [214].

Although human studies conflict with respect to the role of fructose in NAFLD, the totality of the evidence suggests above average fructose consumption is a risk factor for NAFLD in the context of eucaloric and hypercaloric diets [215], and reduction of fructose, albeit accompanied by similar reduction in glucose, can reduce liver fat with minimal weight reduction [216]. The association of saturated

fat intake and IHTAG is supported by an intervention reporting higher IHTAG in a cohort fed a high saturated fat vs. a high n-6 polyunsaturated fat diet [217]. Interventions providing an increased intake of n-3 polyunsaturated have also reported liver fat reductions [218-220]. However, interventions to reduce liver fat demonstrate that a broad range of dietary compositions, including very high fat low-carbohydrate diets, are effective in the reduction of liver fat provided the diet is hypocaloric (see *Chapter 2* for detailed discussion of these interventions).

A comparison of eucaloric, hypercaloric, and hypocaloric diets of different carbohydrate and fat contents each consumed for five days, showed dose-dependent elevation in hepatic *de novo* lipogenesis following carbohydrate surplus and an absence of *de novo* lipogenesis with carbohydrate restriction [221]. Caloric surplus from fat had no effect on hepatic *de novo* lipogenesis. Elevation of hepatic *de novo* lipogenesis after carbohydrate excess was also shown after one [222], and four days [222,223]. A very low fat high carbohydrate eucaloric diet has also been shown to promote whole-body fat synthesis relative to high fat moderate carbohydrate diet [224]. However, these studies were done in healthy and mostly young volunteers given diets only likely to be consumed in a research setting, and with no measures of liver fat reported.

Recently, overfeeding with sugar of patients, 56% of whom had NAFLD, was shown to result in mean liver increasing from 9.2% to 11.7% (27% relative increase) alongside a 2% body weight increase [225]. However, a 90% relative increase in liver fat has also been achieved with four days of a high fat hypercaloric diet [226], but in healthy young males without NAFLD and exact mean IHTAG unspecified . This increase was attenuated by doubling the protein content of the diet despite a resulting further ~15% increase in energy intake. Such a liver fat modifying effect is supported by reports of reduced liver fat following whey protein or amino acid supplementation in NASH patients [227], in obese women with elevated liver fat [228], and elderly patients [229]. It is also supported by a fructose overfeeding trial in young healthy males wherein supplementation with ~20 g of essential amino acids attenuated the increase in IHTAG induced by fructose overfeeding [230].

The majority of studies assessing the relationship of NAFLD and NASH, both prospectively and retrospectively, have had small sample sizes. Retrospective assessment has relied on dietary intake at a single time point. Further, the accuracy of even reference standard dietary assessment is recognised as being poor [231]. Nonetheless, the more tightly controlled studies reviewed above and in Chapter 2 demonstrate that macronutrient composition plays a role in liver fat accumulation, but one that is not yet clearly defined and is likely to be secondary to actual energy balance.

1.7.5 Physical Inactivity and Low Fitness

Physical activity and exercise are inversely and independently correlated with direct [232-234], and biomarker indicators of intrahepatic lipid concentrations [234-236]. In a prospective cohort, total leisure time physical activity and anaerobic/ resistance training physical activity were lower in those going on to develop NAFLD [237]. There is also an inverse association between liver fat and cardiorespiratory fitness [238], i.e. peak oxygen consumption (VO_{2peak}). Indeed, NAFLD was found to be ~10-times more prevalent in the lowest relative to the highest tertile of cardiorespiratory fitness independent of BMI, though not independent of waist circumference [239]. In a cohort of over 72000 Korean adults exercising regularly, defined as more than three times per week for at least 30 minutes over the preceding three months, was associated with reduced odds or having NAFLD independent of BMI [234]. Among the subset of adults with NFALD, those exercising regularly had reduced odds of elevated ALT and AST. Retrospective analysis of a cohort with NAFLD also showed those meeting US guidelines for vigorous exercise to be less likely to have NASH [240]. However, no such association was shown for moderate-intensity or total exercise. Self-reported physical activity was lower in patients with NASH than simple steatosis [208]. Further, cardiorespiratory fitness was identified as an independent predictor of liver fat reduction in lifestyle therapy [8].

The corollary to the inverse association of physical activity and fitness with liver fat is that the capacity to perform vigorous activity is impaired in NAFLD, consistent with disease severity [181]. Physical activity interventions indicate a causal link between inactivity and NAFLD, and show that those with the

condition are able to increase their physical activity [241,242]. Exercise only interventions can reduce liver fat and improve markers of metabolic risk independent of weight reduction [243-245], and also improve markers of autonomic function [246] (see *Chapter 2* for further discussion).

1.7.6 Ethnicity

As shown in *Figure 1.6.* above, the ethnic distribution of NAFLD prevalence is not uniform. In Texas, hepatic steatosis was more prevalent in hispanics and least prevalent in those of African ancestry [156]. A higher prevalence among hispanics, and lower prevalence among African Americans has also been reported in other studies in Texas [183], and California [247]. In a US cohort with biopsy proven NASH, being hispanic was associated with more inflammation but less advanced fibrosis; the latter possibly due to younger age [248]. A higher prevalence of NAFLD has also been reported in Indian men living in Connecticut relative to Caucasians of similar BMI and physical activity history[125]. This effect of ethnicity was confirmed by a multinational study with over 4500 male and female patients showing an influence of ethnicity on the association of body fat distribution and liver fat [249]. This is also consistent with a relatively higher cardiometabolic risk at lower BMI in Southeast Asian and Indian populations related to a tendency for greater body fat and less favourable body fat distribution in these groups at a given BMI [250]. There is evidence of lifestyle differences contributing to this observed variation in risk and aetiology, e.g. hispanics with NASH were younger and reported higher carbohydrate intakes and less physical activity than other groups [248]. However, to what extent differences in lifestyle influence these apparent relationships is not yet firmly established. These findings suggest ethnicity is related to the prevalence of NAFLD, and may also influence its aetiology.

1.7.7 Genetic Factors

The heritability of hepatic steatosis was estimated to be 0.27 [251], and there is evidence to indicate the ethnic differences may have a genetic component [252]. The most consistently identified genetic contributor to NAFLD being the PNPLA3 gene [253], which encodes the adipose tissue associated protein

adiponutrin [254]*.* The precise function of this protein remains unclear [253], however homozygous carriers of the I148M allele of PNPLA3 show markedly higher liver fat than non-carriers [252,255]. Consistent with the ethnic differences in NAFLD prevalence, the I148MM allele was most prevalent in hispanics, whereas a protective gene variant – PNPL3-S453I – was most prevalent in African Americans [255].

Genetic factors appear not only to contribute to the risk of hepatic steatosis but also to its phenotype, progression and comorbidities. Despite achieving similar weight reduction following short-term dietary restriction, liver fat reduction was approximately halved in patients homozygous for I148M relative to those carrying the 148MM allele [256]. Further, in patients with the 148II genotype, the increase in liver fat following sugar overfeeding was closely associated with changes in the fatty acid composition of serum triacylglycerols, whereas no such association was observed in patients with the 148MM genotype [225]. Similarly, the relationship between NAFLD and broader metabolic disfunction, notably insulin resistance, was weaker in SNP rs738409 [257]. The I148M allele is associated with an increased risk of NASH development and fibrosis [258-260]; this may be independent of other metabolic abnormalities [259].

Collectively these findings suggest not only a diverse risk profile based on genetic factors, but also the likelihood of relatively low and high responders to different interventions intended to reduce liver fat. However, to date study cohorts have mostly been small and further work is needed to confirm these findings.

1.7.8 Endocrine Abnormalities

In addition to factors listed in *Table 1.2*, the prevalence of NAFLD is increased in people with several metabolic and endocrine dysfunctions, most notably type 2 diabetes [5]; metabolic syndrome [2,121]; polycystic ovary syndrome [261]; women with a history of gestational diabetes [262]; clinical and subclinical hypothyroidism [263,264]; and low testosterone in men [265].

1.8 Progression, Comorbidities and Sequelae of Non-Alcoholic Fatty Liver Disease

Simple steatosis can progress to NASH characterised by excess liver fat in the presence of inflammation, and subsequently liver fibrosis, cirrhosis, and/or hepatocellular carcinoma [1]. Reports of progression from simple steatosis to NASH have been as high as 37% over 13.7 years [266], but studies to date have been very small with samples sizes of 6-170 and follow-up periods of 3-20 years; [5,267]. Given the heterogeneity of patient characteristics involved, much larger cohorts are required to obtain a clear picture of quantitative disease progression. Progression from simple steatosis to end stage liver disease is estimated to be less than 3% [5].

The largest longitudinal study to date, with a 15 year follow-up, reported 7% of deaths attributed directly to liver disease in a NAFLD cohort [268]. This study was conducted in a general population but did not employ direct measures of steatosis or disease stage, instead relying on composite waist circumference, plasma GGT and TAG concentrations, and is therefore at high risk of misdiagnosis (see Section 1.4.5). Other studies employing more rigorous methods of NAFLD assessment, specifically biopsy or imaging, have reported broadly similar findings, but have had smaller cohorts, shorter follow-up periods, and mostly been in patients drawn from secondary care, and therefore are not likely to be fully representative of the broader community [5,269].

Despite relatively low rates of progression to advanced liver disease, non-hepatic comorbidities are common in cohorts with NAFLD as are deaths attributed to cardiovascular disease and non-hepatic cancers [268,270]. Thus given the high prevalence of NAFLD (see *Section 1.5*), even the seemingly low rate of liver disease progression represents a large clinical burden. However, the concern from an individual patient perspective is more likely to be that related to the comorbidities and sequelae described below.

1.8.1 Metabolic Syndrome

NAFLD has been described as the hepatic expression of metabolic syndrome because it is frequently associated with the same cluster of central obesity,

dyslipidaemia, insulin resistance, and hypertension [271]. Several groups have developed their own set of criteria for diagnosing the metabolic syndrome as shown in *Table 1.3* [272,273]. None of these definitions include hepatic steatosis, however cross-sectional data indicating that NAFLD is a better predictor of insulin resistance, oxidative stress, and endothelial dysfunction than the ATP III criteria for metabolic syndrome (*Table 1.3*), has led some to suggest NAFLD be added [2]. In practice, this close association of conditions means that most patients with NAFLD will need treatment for multiple comorbidities, which in the case of components of the metabolic syndrome, are all receptive to lifestyle intervention [274-280](discussed in greater detail in *Chapter 2* and *Chapter 3*).

Table 1.3: Criteria for Metabolic Syndrome

WHO (1999)*	NCEP ATP III (2001)†	Joint (2009)‡
Impaired glucose tolerance or type 2 diabetes and/or insulin resistance combined with two or more of the following:	Three or more of the following:	Three or more of the following:
Waist–hip ratio (and/or BMI > 30 kg/m^2): men > 0.9 women > 0.85	Waist circumference: men > 102 cm women > 88 cm	Elevated waist circumference (population and country specific)
Triacylglycerols ≥1.7 mmol/L or treatment thereof	Triacylglycerols ≥1.7 mmol/L	Triacylglycerols ≥1.7 mmol/L or treatment thereof
HDL-cholesterol: men < 0.9 mmol/L women < 1.0 mmol/L	HDL-cholesterol: men < 1.03 mmol/L women < 1.29 mmol/L	HDL-cholesterol: men ≤ 1.0 mmol/L women ≤ 1.3 mmol/L or treatment thereof
Blood pressure ≥ 140/ 90 mm Hg	Blood pressure ≥ 130/ ≥ 85 mm Hg	Blood pressure ≥ 130/ ≥ 85 mm Hg) or anti-hypertensive therapy
Fasting glucose ≥ 5.6 mmol/L	Fasting glucose ≥ 5.6 mmol/L or treatment thereof	Elevated fasting glucose 5.6 mmol/L or treatment thereof

*World Health Organization - definition for elevated fasting glucose was modified in 2001 from 6.1 to that shown [273]
† National Cholesterol Education Program – Third Adult Treatment Panel [281]
‡ Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity [272]
Abbreviations: BMI, body mass index; HDL, high density lipoprotein.

1.8.2 Type 2 Diabetes

The association between NAFLD and type 2 diabetes appears bidirectional in that elevated liver fat is a risk factor for type 2 diabetes and *vice versa* [282]. Repeated detection of NAFLD by ultrasound even non-obese and non-diabetic men was associated with substantially elevated 5-year odds for the development of type 2 diabetes [283]. The presence of type 2 diabetes with NAFLD increases the risk of NASH [284], as well as hepatic fibrosis, cirrhosis, portal hypertension, and hepatocellular carcinoma [285]. Whereas, the presence of NAFLD alongside impaired glucose tolerance independently (of age, sex, BMI, blood lipid profile, and smoking status) predicted development of type 2 diabetes [282,286]. There is a clear inherited component whereby family history of type 1 or 2 diabetes predicts NASH and fibrosis even in those without diabetes [287]. Despite findings to date, the mechanisms of the association between NAFLD and type 2 diabetes remain to be firmly established and not all phenotypic expressions of NAFLD appear causally linked with type 2 diabetes [3].

1.8.3 Cardiovascular Disease

Longitudinal studies indicate cardiovascular complications may account for up to 45% of deaths in cohorts with NAFLD [268,269]. Given the close association of NAFLD with major cardiovascular risk factors such as metabolic syndrome and type 2 diabetes, discussed above, this comes as no surprise. However, there is evidence to suggest NAFLD may be an independent risk factor for cardiovascular disease [4,288]. Intima-media thickness was higher in patients with ultrasound confirmed NAFLD than controls without signs of hepatic steatosis [289-292]. This was also true for coronary artery calcification [293]. Intima-media thickness was found to increase with severity of NAFLD [294]. Further, left ventricular diastolic dysfunction in a cohort with type 2 diabetes was also associated with NAFLD [295]. These associations between NAFLD and heart diseases or markers of heart disease, were independent of traditional risk factors including age, sex, BMI, plasma lipid profile, insulin resistance, smoking status and blood pressure [291-294]. However, disease severity, NASH vs. simple steatosis, was not associated with an increased risk of cardiovascular disease in another cohort once results were controlled for age, sex, BMI, and the

presence of type 2 diabetes [296]. These studies suggest that the presence of NAFLD increases the risk of cardiovascular disease beyond that explained by traditional risk factors. However, the magnitude of this increased risk and the exact mediators remain unclear.

1.8.4 Cancer

Cancer contends with cardiovascular disease as the leading cause of mortality in cohorts with NALFD with 28-36% of deaths related to non-liver cancers [204,268,297]. Ultrasound detected NAFLD has been identified as an independent (of age, sex, BMI, and glucose tolerance) risk factor for colorectal neoplasia [298]. NAFLD was associated with risk of colorectal adenomas independent of age, sex, BMI, blood lipid profile, fasting glucose, and smoking status [299]. Patients with NAFLD also had more adenomas than controls showing no hepatic steatosis on ultrasound [300]. However, the presence of NAFLD did not change prognosis in patients with colorectal cancers [301]. Thus although data on mortality clearly show an association between NAFLD and cancer, more work is required to define the nature of this relationship.

1.8.5 Kidney Disease

NAFLD was an independent – of age, sex, diabetes duration, BMI, components of metabolic syndrome – predictor of chronic kidney disease in patients with type 1 [302,303], and type 2 diabetes [304], as well as non-diabetic Korean men [305]. The annual incidence of chronic kidney disease in one cohort of patients with NAFLD was 1.2% [306]. The research in type 1 and 2 diabetes is restricted to cross-sectional analysis of relatively small geographically collocated samples [302-304]. The only prospective study indicated fatty liver only increases risk of chronic kidney disease in the presence of elevated serum GGT [305]. However, this study was restricted to non-diabetic normotensive Korean males. Elevated serum GGT was also identified as a predictor of chronic kidney disease development in Japanese patients with fatty livers [306]. Collectively, these results justify further investigation in other ethnically, and geographically diverse prospectively assessed cohorts, to confirm the suggested associations.

1.8.6 Altered Drug Metabolism

In addition to predicting and potentially being part of the pathology of the aforementioned conditions, and altering nutrient metabolism (see *Section 1.2*), non-alcoholic fatty liver has also been shown to affect expression and activity of many enzymes central to drug and other xenobiotic metabolism [307-309]. Human data are limited due to the difficulties in accurately identifying and staging NAFLD and obtaining liver samples (see *Section 1.4*). Nonetheless, there is evidence from biopsy samples showing changes in messenger RNA expression, protein concentration, and/or in enzymes involved in the metabolism of the vast majority of therapeutic drugs including those of the cytochrome P450 family, glutathione, and sulfotransferases [307].

The direction of change is not universal with some enzymes showing increased and others decreased activity [310], which would be expected to increase and decrease drug clearance rate, respectively. The degree of change tends to increase with disease progression [310]. Although not confirmed, several mechanisms have been suggested for these changes; notably NAFLD associated changes in inflammatory mediators, nonesterified fatty acids, and oxidative stress [307]. The clinical implications of these changes remain unclear but are noteworthy as direct assessment of hepatic steatosis is not a routine practice in drug trials.

1.9 Summary

NAFLD encompasses a diverse spectrum of liver conditions ranging from simple steatosis, through steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. A diagnosis of NAFLD requires alcohol, hepatotoxic drugs, hepatic viral infection, and other common hepatic morbidities have been ruled out. Nonetheless, NAFLD remains a broad term encompassing a range of phenotypes and genotypes, which require further characterisation.

Defined on the basis of hepatic steatosis, NAFLD affects 20-40% of the general population. The prevalence is higher among certain ethnic groups, the obese, and those with type 2 diabetes. There is a close association between NAFLD and the components of the metabolic syndrome, type 2 diabetes, and cardiovascular disease. Emerging evidence also suggests an independent association of NAFLD with kidney disease as well as some non-hepatic cancers, most notably of colon.

Altered nutrient and drug metabolism are hallmarks of NAFLD. Most notably hepatic lipid and glucose production are markedly increased. Correction of these metabolic derangements are recognised targets for treatment. Modifiable risk factors for NAFLD include insulin resistance, adiposity, sedentariness, and hypercaloric diets. Modification of these risk factors using diet and physical activity interventions is the currently recommended treatment approach as well as the focus of this thesis.

The rest of this thesis comprises of five more chapters and a set of electronic appendices provided on an accompanying CD.

Chapter 2 is a systematic review of interventions in NAFLD cohorts that have employed nutrition, physical activity, exercise, or a combination thereof.

Chapter 3 is an assessment of the safety, tolerability, and efficacy of high intensity intermittent training in healthy and clinical populations sharing characteristics with NAFLD patients.

Chapter 4 provides the methods and results of a cross-sectional analysis examining the associations of age and physical activity with cardiorespiratory fitness, glucose control, insulin sensitivity, body composition, and intrahepatic lipid concentration.

Chapter 5 provides the methods and results of parallel design randomised controlled trial assessing the effect of high intensity intermittent training on glucose control, intrahepatic lipid concentration, and body composition in an adult population with clinically defined NAFLD.

Chapter 6 provides a discussion of the broader findings of this thesis, and implications of those findings.

Chapter 2. Systematic Review of Lifestyle Interventions for the Treatment of Non-alcoholic Fatty Liver Disease in Adults

2.1 Introduction

Given the common risk factors and comorbidities, and in the absence of approved pharmacotherapy, lifestyle modifications with the aim of weight reduction and increased physical activity are the recommended treatment for NAFLD [14,311]. However, until recently, the evidence base for this recommendation had not been systematically reviewed. The following section is a systematic review of lifestyle interventions for the treatment of NAFLD. The review was published in the Journal of Hepatology [15], and updated in February 2012 for this thesis. The following sections define, in adults, the:

1. Efficacy of different lifestyle interventions in reducing IHTAG and/or liver aminotransferases;
2. Effect of lifestyle interventions on histological parameters; and
3. The efficacy of different lifestyle interventions on glucose control/ insulin sensitivity.

2.2 Methods

2.2.1 Eligibility Criteria

The review is restricted to published prospective interventions reporting the effects of lifestyle modification on IHTAG, liver enzymes, and/or insulin sensitivity in adults (≥19 years) with NAFLD, including non-alcoholic steatohepatitis (NASH) but not late stage liver diseases i.e. cirrhosis or hepatocellular carcinoma. Eligible publications included: randomised controlled trials or specific arms thereof, and non-randomised interventions. Only full reports were considered to provide sufficient information to allow critical evaluation.

No specific criteria defining NAFLD were set as the methods of diagnosis and cut-offs vary between studies. It was considered sufficient for reports to provide their own diagnostic criteria based on one or more of the following in order of preference: 1) histological examination of biopsies; 2) proton-magnetic resonance spectroscopy (^{1}H-MRS); 3) computed tomography (CT); 4) ultrasound; and/or 5) blood concentrations of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST).

Lifestyle modification could include general recommendations or specific diet, physical activity, and/or exercise prescription. Studies or study arms designed to test pharmaceuticals, dietary supplements, or herbal preparations were excluded. Study arms in which pharmaceutical agents were used as part of standard treatment and where participants were receiving these prior to the study, without a reported increase in dose during the study, were eligible for inclusion.

The primary outcomes of interest were changes in IHTAG assessed by liver biopsy, ^{1}H-MRS, CT, or ultrasound, and histological indicators of inflammation and fibrosis. Blood ALT and/or AST concentrations were also considered. The secondary outcome was glucose tolerance and/or insulin sensitivity as assessed directly by insulin clamp techniques, oral glucose tolerance tests, or inferred by validated formulae.

Only studies that clearly described or appropriately referenced their intervention, and that provided some direct indicators of protocol adherence or those conducted under very close supervision, e.g. inpatient protocol delivery, were eligible for inclusion.

2.2.2 Search Strategy and Study Selection

The following databases were searched: Medline (Pubmed), Scopus, and the Cochrane Controlled Trials Register. The last search of all three databases was done on June 26, 2010. However, automatic updates of the Scopus search were reviewed up to October 18, 2010. A medical librarian assisted with the selection of the search strategies. Further searches were conducted in February 2012 to update this chapter.

The selected search terms and related MESH headings were: (NAFLD or "non-alcoholic fatty liver" or "nonalcoholic fatty liver" or "non-alcoholic steatohepatitis" or "nonalcoholic steatohepatitis" or "non-alcoholic steatosis" or "nonalcoholic steatosis" or "non-alcoholic liver steatosis" or "nonalcoholic liver steatosis" or "non-alcoholic hepatic steatosis" or “nonalcoholic hepatic steatosis”) AND (lifestyle or exercise or "diet*" or diet or training or behaviour or behavior or nutrition or sport or "physical activity" or "weight reduction" or "weight loss" or "energy restriction"). These were restricted to title, abstract, and keyword (Scopus only). The database permitting, the following were excluded: reviews; letters; editorials; commentaries; animal studies; and studies in those aged under 19 years. Review of articles was restricted to those published in English.

Titles and abstracts of studies identified were evaluated against eligibility criteria. Studies appearing eligible based on their abstract were read in full.

2.2.3 Data Items

The items of interest from each report included: study type/design; diagnostic criteria for NAFLD; inclusion and exclusion criteria; blinding; similarity of groups at baseline; sex; age; definition of participant adherence; treatment protocol, including professions involved and contact time; reported adherence; criteria for

dealing with medication; methods used to assess diet and physical activity; loss to follow-up; analysis, i.e. intention-to-treat vs. per-protocol; IHTAG; measures of glucose control/insulin sensitivity; ALT and/or AST concentration.

2.2.4 Data Extraction

Relevant data from included reports were recorded in itemised tables. Results were converted to SI units or otherwise standardised, and changes from baseline converted to percentages to facilitate comparison across studies. Where liver fat is given as a percentage, a change from 10% fat to 5% fat is referred to as an absolute reduction of 5% (10%-5%) and a relative reduction of 50%.

Multiple publications from the same study were identified by comparing author names, sample sizes, and intervention protocols. Where papers noted that other reports of the study existed, these were also obtained to allow consistency between different reports to be assessed and/or missing data to be obtained.

2.2.5 Risk of Intra- and Inter-Study Reporting and Publication Bias

Included studies were compared to their published protocols when available to identify omissions of outcome data. Alternatively, the methods section of each report was compared to the results section to assess reporting bias. The International Clinical Trials Registry, EU Clinical Trial Register, and metaRegister of Controlled Trials were searched using the key words fatty liver and steatohepatitis to identify trials described as completed. Studies registered prior to 2009 with records not updated in the past 12 months were assumed to be completed. A literature search using the relevant principal investigator was conducted to identify publications resulting from relevant registered trials.

2.2.6 Description and Critique of Primary Outcome

A description and critique of methods used in the analysis of hepatic steatosis is provided in *Section 1.4* of this thesis.

2.3 Results

A flow diagram of study selection consistent with *Preferred Reporting Items for Systematic Reviews* is provided in Figure 2.1 [312].

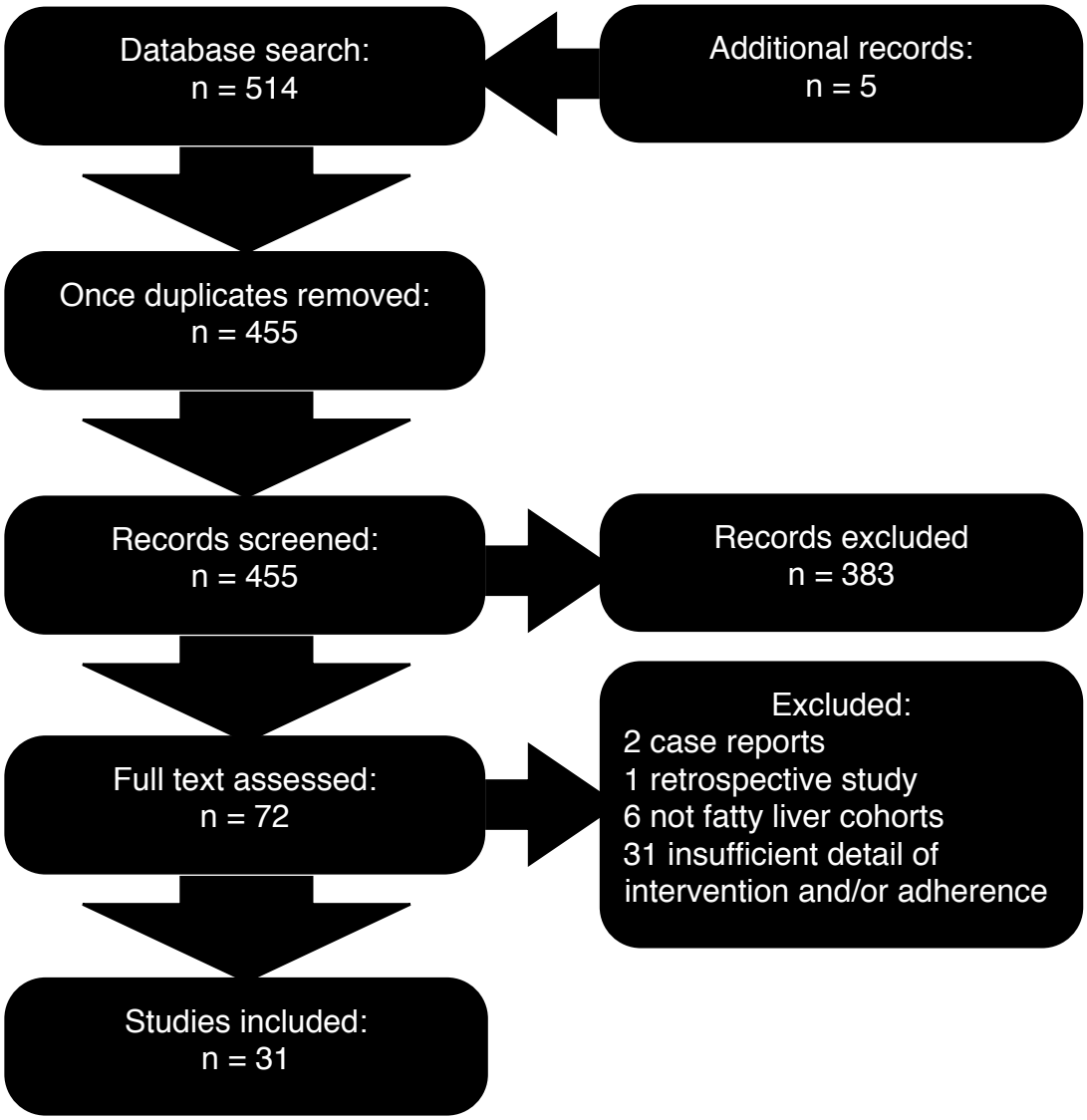


Figure 2.1: Diagram of Study Selection. NB: one dataset was analysed in multiple ways published in two papers but is counted as one study.

2.3.1 General Limitations of Studies Reviewed

Specific details about individual studies reviewed can be found in Tables 2.1-2.4. There was considerable heterogeneity of assessment methods used, diagnostic criteria for NAFLD applied, and the detail with which exclusion criteria were reported. With one exception, studies employing ^{1}H-MRS either did not report a minimum IHTAG or report including participants <5%. Those employing histological examination of biopsies applied cutoffs, but used

different scoring systems. Eligibility based on history of alcohol consumption varied between no alcohol intake and 560 g/week, and assessment methods were seldom cited. Although most studies noted excluding participants with other liver conditions, including potential drug induced steatosis, few provided comprehensive criteria, e.g. of drugs deemed steatogenic, or details of the relevant analytical methods employed.

Monitoring of adherence to diet or exercise was often limited. No studies reported using objective measures of physical activity such as accelerometers, instead questionnaires were used. Dietary assessment methods were not reported in sufficient detail to assess likely accuracy and, except for β-hydroxybutyrate in ketogenic diets, no biomarkers of adherence were reported. The greater the complexity of interventions, the less precisely the actions of participants tended to be reported. Interventions involving diet, exercise, and behaviour change methods were reported with a focus on outcomes, and limited information on diet and physical activity adherence or how behaviour change methods were applied. None of the reviewed papers reported an assessment of which particular behaviour change techniques accounted for the changes reported.

Studies did not report allocation concealment during data analysis except for blinding during the analysis of liver biopsy samples. Some studies doing per protocol analysis did not provide baseline data for the specific group in the final analysis. Most studies did not have a control group; those that did, provided this group with some form of limited intervention.

2.3.2 Study Findings

The studies identified fell into four broad categories: those involving only dietary intervention; exercise only interventions; and combined interventions with either broad physical activity recommendations; or specific exercise prescriptions. A further subcategory would have been those employing one or more formal models of behaviour change. However, to-date, no study has published sufficient data to critically evaluate the application of different theories, or

determine which aspects of each theory are most closely associated with particular disease outcome measures or biomarkers.

2.3.2.1 Diet Only Interventions

Fourteen studies, summarised in Table 2.1, lasting 2-26 weeks and including 428 participants (approximately ⅔ women) that described diet only interventions were identified: 10 using low-to-moderate fat (≥30% of energy) with moderate-to-high carbohydrate (≤50% of energy) energy restricted diets [171,173,313-319], one of which also specifically restricted iron intake [314]; four interventions assessed low carbohydrate ketogenic diets [171,173,320,321]; and four high protein (≥25% of energy) diets [173,318,322,323]. Two studies employed biopsies [314,320], but only one at follow-up [320], the other study used ALT and AST at follow-up [314]; seven used ^{1}H-MRS [171,173,315,317-319,322], two used CT [313,321], three studies relied on ALT and AST [316,323,324]. Only two studies had a no-intervention/standard care control group [313,314]; however in one the control group were those with low adherence to the intervention protocol [313].

Three studies compared hypocaloric diets focusing on fat vs. carbohydrate reduction [171,173,319]. Carbohydrate restriction to 10% of energy in a diet with a 1000 kcal/day deficit led to a much greater mean reduction in IHTAG than an isocaloric 65% carbohydrate diet within 48 hours (approximately 30% vs. 10%) [171]. Greater mean reductions in IHTAG (55% vs. 26%) were also reported after two weeks of low carbohydrate (8% of energy) vs. moderate carbohydrate and fat diet (50% and 34% of energy, respectively) [173]. However, after several weeks and with similar weight reductions in low carbohydrate and low-to-moderate fat, IHTAG reductions were similar [171,319].

Interventions lasted 2 weeks to 6 months and achieved mean bodyweight reductions of 4-14%. All studies using biopsy or imaging techniques to estimate IHTAG reported reductions. The seven studies using ^{1}H-MRS reported absolute reductions of 4 -12%, and relative reductions of 26 - 81%. The only study to do a post intervention biopsy (n = 5) reported reduced inflammation and a trend toward reduced fibrosis (p =0 .07), as well as the reduction in steatosis, following a ketogenic diet and a mean weight reduction of 14% [320]. Five out of

seven studies reporting liver enzymes showed reductions, and one showed no change. The study that found an increase in ALT and AST, but only in women, suggested this might have been due to the analysis being done before weight had stabilised [323]. Five of six studies reporting glucose control/insulin sensitivity noted improvements.

Table 2.1: Diet Only Interventions

Reference & Design	n (M/F)	Clinical Group	Age (years)	BMI (kg/m²)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(171) RT	11(n/r)	NAFLD	45±4	37±1	**High CHO:** (65% CHO 20% fat, 15% PRO): 1000 kcal/d below estimated (RMR x 1.3) until 7% BW reduction achieved at wk 6.2±1.0, then E adjusted to maintain BW until week 11	11	IHTAG (^{1}H-MRS), E-HC: HISI, GR_a, HOMA	Relative to baseline: **At 48 h** (BW ↓1.6%): IHTAG ↓~10%, HOMA ↓24±6%, HISI ↑~35%, GR_a ↓~7%. **At 11 wk** (BW -7.3%): IHTAG ↓~42%, HOMA ↓27.1±5.1%, HISI ↑~28%, GR_a ↓~7%.
	11(n/r)	NAFLD	42±3	36±1	**Low CHO**: (10% CHO, 75% fat, 15% PRO): 1000 kcal/d below estimated (RMR x 1.3) until 7% BW reduction achieved at wk 5.9±1.0, then E adjusted to maintain BW until wk 11	11	Food provided by metabolic kitchen. Plasma 3-hydroxybutyrate indicated good adherence to low CHO diet	Relative to baseline: **At 48 h** (BW ↓2.2%): IHTAG ↓~30%, HOMA ↓40%, HISI ↑~140%, GR_a ↓23%. IHTAG, HISI, HOMA, GR_a were all significantly different between groups. **At 11 wk** (BW -7.6%): IHTAG ↓~38%, HOMA ↓44%, HISI↑~115%, GR_a ↓~20%. IMGU was reported for both groups combined: no change after 48 hrs, ↑~30% after 11 weeks.
(320) Pilot	5(2/3)	NAFLD	35.6	102±12 kg	Ketogenic diet: <20 g CHO/d, unlimited meat/fish/poultry, unlimited eggs, 2 cups/d 'salad' vegetables, 133 g cheese, 1 cup/d low-CHO vegetables.	24	Histopathology, ALT, AST Diet records & urinary ketones indicated good compliance in 4 participants. No change in non-compliant participant. Actual intake n/r.	BW ↓14% Adherent (n=4): steatosis, necroinflammation & centrilobular fibrosis ↓1-2 categories (e.g. moderate to mild or none), ALT ↓44%, AST n.s. Non-adherent (n=1): steatosis ↑ from mild to moderate, ALT ↓20%, AST ↓21%.
(322) UCT	34(10/24)	NAFLD (~50%)	n/r	31±1	6 wk of 550 kcal/d diet from ready made product (50% CHO, 7% fat, 43% PRO), then 1 wk eucaloric diet before final assessments.	7	IHTAG (^{1}H-MRS), EGP (euglycaemic clamp with radiolabelled glucose), HbA_{1c}	BW ↓11% IHTAG ↓60%, IS ↑32%, EGP ↓34%, HbA_{1c} ↓3.6%.

Reference & Design	n (M/F)	Clinical Group	Age (years)	BMI (kg/m^2)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(315) UCT	8(5/3)	T2D	47±3	30±1	Liquid formula low fat diet (50% CHO, 3% fat, 43% PRO) + 12 g dietary fibre + raw fruit & vegetables to ~ 1200 kcal/d. Diet continued until normoglycaemia, then 4 wk maintenance: 35 kcal/kg/d (60% CHO, 20% fat, 20% PRO)	7-12	IHTAG (^{1}H-MRS), E-HC: HISI, GRa, IMGU, IMSGP, HOMA Weekly checkup visits including weigh ins & provision of food during study.	BW ↓8% IHTAG ↓81±4% (to 2.2±0.8%), GRa ↓~18%, IMGU n.s., IMSGP ↑221% (to 93±5%)
(321) UCT	14(5/9)	Pre-bariatric surgery	24-45	46 (40-57)	CHO restriction to 30 g/d but no other restriction under weekly supervision of a dietician + resources on the CHO content of vegetables & dairy foods	4	CT: liver:spleen ratio & liver volume; ALT, AST & fasting glucose. Daily food dairy indicated: 1520±285 kcal/d (1109-1922 kcal/d) (14% CHO, 56% fat, 29%).	BW ↓3.7%. Liver:spleen ↑16% (p=0.06); liver volume ↓8.2% (223 mL); ALT, AST, & glucose n.s.
(313) UCT	17(6/9)	NAFLD	47±3	33±1	**Adherent:** dietary deficit of 500-1000 kcal/d (55% CHO, 30% fat, 15% PRO) (weight ↓≥5%)	26	Liver density (CT); ALT, AST, HOMA. Food dairy reported ↓641 cal/day (47% CHO, 33% fat, 20% PRO)	Change relative to baseline: BW ↓9.4 Adherent: ALT ↓28%, AST n.s., liver density ↑15%, HOMA ↓42%.
	14(7/7)	NAFLD	47±3	35±2	**Non-adherent** as above but BW↓<5%)		Liver density (CT); ALT, AST, HOMA. Food dairy reported ↓479 kcal/d (48% CHO, 31% fat, 21% PRO)	Change relative to baseline: BW ↓1.8% ALT, AST, liver density & HOMA n.s

Reference & Design	n (M/F)	Clinical Group	Age (years)	BMI (kg/m^2)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(314) RCT	12(n/r)	9 NASH 3 NAFLD†	58±3	26±1	**Diet:** 30 kcal/kg BW/d (20% fat, 1.1-1.2 g PRO/kg BW/d, iron <6 g/kg BW/d)	26	ALT & AST. 3-d diet records: At 3 months: 30 kcal/kg BW/d (20% fat, 6.1 g iron/kg BW/d, 1.3 g PRO/kg BW/d), serum ferritin ↓42%. At 6 months: 25 kcal/kg BW/d (20% fat, 5.3 iron/kg BW/d,1.2 g PRO/kg BW/d), serum ferritin ↓54%.	Relative to baseline: 3 months: BW ↓3.2%, ALT ↓45% & AST ↓34%. 6 months: BW ↓4.8, ALT ↓60%, AST ↓52%.
	6(n/r)	2 NASH 4 NAFLD†			**Control:** no intervention	26	n/r	at 6 months: BW n/r) ALT & AST n.s.
(323) UCT	147 (33/104)	Obese + elevated ALT	44 20-69	38±0	**Diet**: 800 kcal/d soy-based meal replacement (38% CHO, 17% fat, 45% PRO)	8	ALT & AST	BW ↓11% Men: ALT ↓21%, AST ↓13% Women: ALT ↑52%, AST ↑32%
(324) UCT	30(8/22)	Obese + elevated ALT	44±3	37±1	**Diet**: 1520 kcal/d (52% CHO, 25% fat, 23% PRO)	12	ALT, AST, HOMA Diet (3-d diet records): 1509±373 kcal/d (40% CHO, 39% fat, 21% PRO)	BW ↓4.6% ALT ↓28%, AST ↓48%, HOMA ↓39
(316) UCT	14(7/7)	NAFLD by US	45±1	28±0	**Diet**: 25 kcal/kg of ideal BW (1.3 g PRO/kg; 20.8% energy, 0.7 g of fat/kg; 25% E, 3.4 g of CHO/kg; 54.2% E, PUFA:SAFA ratio 1.0–1.5; n-6:n-3 PUFA ratio 3.0–3.5) rich in fish & vegetables & low in meat.	24	ALT & AST (3-d diet records): 28.6 kcal/kg ideal BW/d (56% CHO, 28% fat, 17% PRO), PUFA:SAFA 0.8:1, n-6:n-3 2.4:1.	BMI ↓3.6%, ALT ↓52%, AST ↓42%

Reference & Design	n (M/F)	Clinical Group	Age (years)	BMI (kg/m^2)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(173) RCT‡	9(3/6)	NAFLD	47±12	34±9	**Low E**:1200 kcal/d for women, 1500 kcal/d for men; prepared by metabolic kitchen based on individual food records	2	IHTAG (^{1}H-MRS), ALT, AST, fasting glucose Diet (7-d diet record): 1325 kcla/d (50% CHO, 34% fat, 16% PRO).	BW ↓4.2% IHTAG ↓26% (less than low CHO group), AST ↓73%, ALT n.s., fasting glucose n.s.
	9(2/7)	NAFLD	42±11	36±4	**Low CHO**: target >20 g CHO/day; 7 d self-administered + 7 d prepared by metabolic kitchen	2	IHTAG (^{1}H-MRS), ALT, AST, fasting glucose Diet (7-d diet record): 1553 kcal/d (8% CHO, fat 59%, 33% PRO).	BW ↓5.2% IHTAG ↓55% (greater than in low E group), AST ↓66% (n.s. relative to low E group), ALT n.s., fasting glucose n.s.
(317) UCT	25(15/10)	21 NAFLD	57±8	33±4	**Diet**: 597 kcal/d below maintenance & defined as low fat but no % was specified.	16+6*	IHTAG (^{1}H-MRS), HOMA **Diet** (2 x 24 hr record): 1405 kcal/d (50% CHO, 26% fat, 21% PRO).	BW ↓6% IHTAG ↓29% HOMA ↓16%
(318) UCT	15(0/15)	Pre-bariatric surgery	34±8	43±3	**Diet**: 800-1100 kcal/d from Modifast® (45% CHO, 15% fat, 40% PRO).	4	IHTAG (^{1}H-MRS) Diet: food provided as liquid supplement - Modifast®	BW ↓6% IHTAG ↓42%
(319) RCT	20(15/5)	NAFLD	45±8	36±5	**Low CHO**: baseline E intake -30% (minimum 1200 kcal/d): ≤90 g CHO/d; 0.8 g PRO/kg/d; ≥30% fat	26	IHTAG (^{1}H-MRS) Bimonthly 7-day food record: E & CHO ↓n.r. (graphic data) relative to baseline & Low Fat	BW ↓8% IHTAG ↓50%
	27(8/19)	NAFLD	46±9	34±4	**Low Fat**: baseline E intake -30% (minimum 1200 kcal/d): ≤20% fat, 0.8 g PRO	26	IHTAG (^{1}H-MRS) Bimonthly 7-day food record: E & Fat ↓n.r. (graph data)	BW ↓7% IHTAG ↓44%

Data are mean ± standard errors usually rounded to the nearest full number; changes reported were statistically significant $p < 0.05$ unless noted otherwise. Sample size based reflects those in the final analysis. Abbreviations: ALT - alanine aminotransferase; AST - aspartate aminotransferase; BMI - body max index (kg/m^2); BW - bodyweight; d - day(s); CHO - carbohydrate; CT – computed tomography; d - day(s); E - energy; EGP- endogenous glucose production; E-HC - euglycaemic-hyperinsulinaemic clamp; GRa - glucose rate of appearance; h - hour(s); HbA_{1c} – glycated haemoglobin; HISI - hepatic insulin sensitivity index; HOMA - homeostasis model assessment; IHTAG - intrahepatic triacyglycerol concentration; IMSGP - insulin mediated suppression of glucose production; IMGU - insulin mediated glucose uptake; IS - whole body insulin sensitivity; ITT – intention to treat analysis; LBM - lean body mass; MUFA - monounsaturated fatty acid; n - sample size of cohort in final statistical analysis; NAFLD - non-alcoholic fatty liver disease; NASH - non-alcoholic steatohepatitis; n/r, data not reported; n.s. – result not statistically significant; PP – per protocol analysis; PRO - protein; PUFA - polyunsaturated fatty acid; RCT – randomised controlled trial; RT – randomised trial with no control; RMR - resting metabolic rate; SAFA - saturated fatty acid; UCT – uncontrolled trial; US – ultrasound; w – week(s).

† Diagnoses by histopathology at baseline only, no repeat biopsies on completion of the intervention.
‡ Semi-randomised to achieve baseline characteristic equivalence between groups, specific measures matched were not reported.
* Weight reduction followed by maintenance.

2.3.2.2 Exercise Only Interventions

Five studies, summarised in Table 2.2, lasting 4-16 weeks and including 107 participants (approximately 50% women) assessed exercise only interventions. Three studies tested moderate intensity continuous aerobic exercise such as walking, running, stationary cycling, and rhythmic movement for 30-60 minutes per session three [244], five [245], and four to six times per week [325]. One study used a circuit resistance training programme covering all major muscle groups and performed three days per week for eight weeks [243]. One study employed electrical stimulation to create resistance during knee flexion and extension twice a week [326].

Moderate intensity aerobic activity resulted in mean relative ^{1}H-MRS-measured IHTAG reductions of 10-21% [244,245], and 47% and 48% reductions in ALT and AST, respectively [325]. Circuit resistance training, also assessed by ^{1}H-MRS, gave a mean relative reduction of 13% [243]. The effect of electrical stimulation exercise was assessed by biopsy, and a reduction in steatosis reported [326]. These results were achieved despite no significant reduction in body weight in exercising groups.

The shorter, 4 week, aerobic intervention reported no change in homeostatic model of insulin resistance (HOMA-IR) [244]. However, electrical stimulation exercise reduced HOMA-IR by 54% after 12 weeks. Circuit resistance training reduced area under the glucose curve of a two-hour frequently sampled oral glucose tolerance test and HOMA-IR by 12% and 22%, respectively [243]. The other two studies did not report measures of glucose control/insulin resistance.

It is noteworthy that the electrical stimulation exercise group was drawn from a population that had previously been given lifestyle intervention counselling, but with minimal change to disease biomarkers [326]. It is feasible therefore that the exercise intervention may have motivated participants to change their lifestyle more in line with previous advice thereby exaggerating the apparent effect of the intervention.

Table 2.2: Exercise Only Interventions

Reference & Design	**Sample (M/F)**	**Clinical Group**	**Age** (years)	**BMI** (kg/m^2)	**Intervention**	**Length** (weeks)	**Outcome Measures & Adherence**	**Results**
(244) RCT	12(n/r)	NAFLD	47±4	31±1	**Aerobic**: 3 supervised cycle ergometer sessions per wk x 4 wk. Weekly progression of VO_{2peak}: 50%, 60%, 70% (wk 3 & 4) for 2-3 bouts of 15 min with 5 min rest between.	4	IHTAG (^{1}H-MRS), ALT, HOMA. Participants attended all 12 sessions.	HTAG ↓21% in intervention group (8.55% to 6.79%); no change in ALT. No change in HOMA-IR, fasting glucose, or insulin within or between groups.
	7(n/r)	NAFLD	49±2	32±2	**CON**: 3 home-based whole body stretching sessions/wk.	4	IHTAG (^{1}H-MRS), ALT, HOMA.	IHTAG, ALT, and HOMA n.s.
(325) UCT	16(n/r)	Elevated ALT	~37†	23±0	**Aerobic exercise**: 45 min/6 d/wk with 60-70% estimated HR_{max} maintained for 20 min/session. Exercise options included walking, jogging, and rhythmic aerobic exercises.	12	ALT & AST	From baseline: ALT ↓47%, AST ↓48%.
(243) RCT	11(9/2)	NAFLD	52±13	32±5	**Resistance training**: 3 session per wk: 8 exercises covering all major muscle groups done in a circuit for 2-3 sets with repetitions increasing from 8-12 over the course of each week.	8	IHTAG (^{1}H-MRS) fsOGTT AUC	IHTAG ↓13% (greater change than CON) fsOGTT AUC ↓12% (greater change than CON)
	8(6/3)	NAFLD	62±7	32±5	**CON**: no intervention, participants advised to keep their current diet and physical activity.	8	IHTAG (^{1}H-MRS) fsOGTT AUC	IHTAG n.s. fsOGTT AUC ↑12% relative to baseline & ↑24% relative to exercise group

Reference & Design	Sample (M/F)	Clinical Group	Age (years)	BMI (kg/m^2)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(326) RCT‡	12(5/7)	NAFLD	55±4	28±1	**Hybrid training**: 2 sessions per week in which antagonist muscle contraction was increased by electrical stimulation during knee flexion & extension.	12	Steatosis grade (US) ALT, AST, HOMA Full attendance of sessions	Steatosis grade ↓ relative to baseline & CON. ALT ↓22% (greater change than in CON), AST n.s. HOMA ↓54% (greater change than in CON)
	23(14/9)	NAFLD	52±4	27±1	**CON**: no intervention, however all participants had been previously given lifestyle advice but without positive change in biomarkers.	12	Steatosis grade (US) ALT, AST HOMA	Steatosis grade, ALT, AST, and HOMA n.s.
(245) RCT	12(4/8)	NAFLD	49±2	37±1	**Aerobic exercise**: 30-60 min moderate intensity continuous aerobic exercise 5/w	16	IHTAG (^{1}H-MRS) ALT Completers did 94% of prescribed exercise	IHTAG ↓10% (relative to CON) ALT ↓13% (relative to CON)
	6(1/5)	NAFLD	48±3	40±2	**CON**: continuation with prior physical activity and dietary habits.	16	IHTAG (^{1}H-MRS) ALT	IHTAG n.s. ALT n.s

Data are mean ± standard errors; changes were statistically significant $p < 0.05$ unless noted otherwise; Abbreviations: 1H-MRS – proton energy magnetic resonance spectroscopy; ALT - alanine aminotransferase; AST - aspartate aminotransferase; BMI - body max index (kg/m2); BW - bodyweight; d - day(s); CON - control group; F, females; fsOGTT AUC - area under the glucose curve from a frequently sampled oral glucose tolerance test; HOMA - homeostasis model assessment insulin resistance; IHTAG - intrahepatic triacylglycerol concentration; M, males; min - minute; n/r – not reported; non-RCT - non-randomised but with a control group; n.s. - not statistically significant change; PP – per protocol analysis; UCT – uncontrolled trial; US - ultrasound; VO2peak – peak oxygen consumption ml/min/kg; wk - week(s).

† Age was only reported for the combined study groups - see Table 4 for other study group.
‡ Treatment allocation by geographic location.

2.3.2.3 Diet Combined with Physical Activity and/or Exercise Advice

Seven studies involving 434 participants (approximately 50% women; 98 controls) employed a selection of behaviour change methods to decrease energy intake and increase physical activity/exercise over 3-12 months [8,241,242,327-331]. These studies provided general physical activity guidelines, but did not prescribe specific exercise protocols. Key study details are summarised in Table 2.3. The focus was predominantly on body weight reduction and maintenance with mean reductions of 2.2-8.8%. Only two studies reported an objective measure of physical activity adherence, specifically changes in cardiorespiratory fitness [8,241,242]. One reported energy intake [241], another stated target energy intakes were achieved but did not report any details [8], and one reported energy intake reductions but not macronutrient composition [331]. Six of seven reported reductions in IHTAG and/or circulating liver enzyme in the intervention groups, with absolute mean reductions in IHTAG, measured by ^{1}H-MRS, of 2-4.6% [8,328]. Relative mean reductions assessed by biopsy [327], CT [329,330], or ^{1}H-MRS were 13-51% [8,328]. Six of the seven studies reported improvements in glucose control//insulin sensitivity [8,241,242,328-330].

Promrat *et al.* assessed histopathology and reported significant ($p \leq 0.05$) reductions in overall NAFLD histological activity score (NAS) and steatosis. But reductions in parenchymal inflammation and ballooning injury were not significant, and there was no mean change in fibrosis in the intervention relative to the control group or baseline [327]. Huang *et al.* also assessed histopathology, but reported only a trend in reductions in hepatitis score ($p = 0.06$) [331].

Table 2.3: Interventions Combining Diet and Broad Physical Activity/Exercise Advice

Reference & Design	Sample (M/F)	Clinical Group	Age (years)	BMI (kg/m²)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(327) RCT	21(14/7)	NASH	49±2	34±1	**LI**: weekly sessions for 6 months then biweekly covering: diet (1000-1200 kcal for <91 kg BW & 1200-1500 kcal for >91 kg with 25% E from fat), PA & exercise (progress to 200 min/w mod intensity) & behaviour modification (stimulus control, problem solving & relapse prevention). Goals: 7-10% weight reduction in 6 months then maintenance.	48	Histopathology (NAS), ALT, AST, HOMA, HbA_{1c} (Actual diet & PA achieved was not reported)	**LI relative to CON:** BW ↓8.8% NASH score ↓26%, steatosis ↓42%; n.s. change in inflammation, ballooning, fibrosis. ALT -39%; n.s. change in AST, HOMA & HbA1c.
	10(8/2)	NASH	48±4	34±2	**CON**: education session on NASH, healthy eating, PA & weight management every 12 wk, but without behaviour change skills training.	48	Histopathology (NAS), ALT, AST, HOMA, HbA_{1c} (Actual diet & PA achieved was not reported)	**LI relative to baseline:** NASH score ↓55%, steatosis score ↓58%; n.s. change in inflammation, ballooning, fibrosis. ALT ↓50%; n.s. change in AST, HOMA & HbA1c.
(331) Pilot	16(8/8)	NASH	50±3	34±1	Weekly (8 wks), biweekly (month 3-6), then monthly visits with dietitian: E restriction n/r (40-45% CHO, 35-40% fat, 15-20% PRO). Weight loss target of 400-800 g/w. Advice to increase PA; frequency & duration n/r.	48	Histopathology (Modified Brunt), ALT, AST, HOMA. E intake ↓195 kcal/d from baseline (food dairy); specific composition n/r. PA ↑ in those with improved NASH Score.	BW ↓3.3% NASH score ↓32% (p=0.06) & ↓47% HOMA p=0.06); steatosis, fibrosis, total NASH score, ALT & AST n.s.

Reference & Design	Sample (M/F)	Clinical Group	Age (years)	BMI (kg/m^2)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(328) RCT	46(n/r)	T2D (33% NAFLD)	61±1	35±1	**LI**: Weekly sessions (group to individual ratio 3:1) for 6 months, then 3 sessions/month at 2:1 ratio for 6 months. Portion control & energy intake targets. Meal replacements in first several weeks. Fat intake <30%. Goal to ↓ BW ≥7% & ↑ moderate intensity PA ≥175 min/wk.	48	IHTAG (^{1}H-MRS), ALT, AST, HbA_{1c} Actual diet & PA of these subgroups not reported.	Relative to baseline: BW ↓8.3% IHTAG ↓51%, ALT & AST n.s., HbA1c ↓8.5%. Changes significantly greater than in CON
	50(n/r)	T2D (54% NAFLD)		35±1	**CON**: standard care + 3 educational group sessions/year.	48		IHTAG ↓23%, ALT & AST n.s., HbA1c n.s.
(8) UCT	50 (28/22)	NAFLD	47±2	32±1	≤10 sessions with a dietitian to: ↓ E intake, particularly from fat & ↑fibre intake. Advice to do 3h/w of moderate aerobic activity.	39	IHTAG (^{1}H-MRS), ALT, AST. VO_{2peak} ↑9.4%, 3-day diet records indicated E and SAFA intake decreased, but quantitative data n/r	BMI ↓3.5% IHTAG ↓35%, ALT ↓18%, AST ↓8.7%, 2 hour glucose ↓11% (75 g OGTT)
(329) RCTS	26(26/0)	T2D	n/r	32±1	**LI**: Weekly sessions (group to individual ratio 3:1) for 6 months, then 3 sessions/month at 2:1 ratio for 6 months. Portion control & energy intake targets. Meal replacements in first several weeks. Fat intake <30%. Goal to ↓ BW ≥7% & ↑ moderate intensity PA ≥175 min/wk.	48	Liver:spleen ratio (CT), E-HC Actual diet & PA of these subgroups not reported.	Men: BW ↓12% Liver:spleen ratio ↑16%, GRd ↑56% (E-HC), fasting glucose ↓18%.
	32(0/32)	T2D	n/r	35±1		48		Women: BW ↓8% Liver:spleen ratio ↑14%, GRd ↑36% (E-HC), fasting glucose ↓6.4%.

Reference & Design	Sample (M/F)	Clinical Group	Age (years)	BMI (kg/m^2)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(241) RCT	38(20/18)	High AST	49±2	32±1	Personalised exercise & diet advice: PA - 150 or 200 min/wk of mod intensity exercise for health or weight reduction, respectively: diet - reduced E by 406-574kcal/day, low in SAFA, high in n-3 fats & fibre. Using: Social Cognitive Theory, Transtheoretical Model, Theory of Self-Determination, and Motivational Interviewing. **MI**: 6 fortnightly sessions. **LI**: 3 counselling sessions in first month. **CON**: 1 baseline session.	12	ALT, AST, HOMA PA ↑124 min/wk, BMI ↓3.2%, E intake ↓266±3 1kcal/day.	Relative to CON: ALT ↓28%, AST & HOMA n.s.
	76(50/26)	High AST	48±2	32±1		12	ALT, AST, HOMA PA ↑115 min/wk, BMI ↓2.2%, E intake n.s.	Relative to CON: ALT ↓22%, AST & HOMA n.s.
	38(25/13)	High AST	47±2	32±1		12	ALT, AST, HOMA **CON**: PA & E intake n.s.	
(242) RCT	Same group as ref 39 divided as described in next column. Baseline data for the post hoc groups were not reported. These analyses do not include the participants with hepatitis C.				Reanalysis of data set from ref 39 on the basis of changes in physical activity relative to controls rather than group allocation: **Increased** - increased PA by ≥ 60 min/week; **Maintained** - maintained baseline PA of >150 min/wk; **Low** - maintained or ↓ PA from baseline at 60-150 min/wk; **Sedentary** - maintained or ↓ PA <60 min/wk.	12	ALT, AST, HOMA As per *Intervention* column.	Results relative to sedentary group: **Increased** (n=85): ALT ↓15 U/L, AST ↓7.6 U/L, 2 h glucose n.s. & HOMA ↓1. **Maintained** (n=29): ALT ↓20 U/L, AST n.s., 2 h glucose n.s., HOMA ↓0.3. **Low** (n=19): ALT ↓15 U/L, AST ↓10 U/L, 2 h glucose & HOMA n.s.
(330) UCT	31(15/16)	NAFLD	55±2	27±1	Home-based lifestyle modification targeting 5% BW reduction: daily weighing, monthly 30 min visit for lifestyle advice, additional nutrition counselling every 3 months, target 25-30 kcal/kg BW/d, 23 MET/wk of PA + 4 MET/wk exercise	12	Liver:spleen ratio (CT), ALT, AST, HOMA	BW ↓8.5%, ALT ↓21%, AST ↓7%, liver spleen ratio ↑13%, HOMA ↓48%.
						24	Diet records were collected but findings n/ r; PA n/r.	(from baseline n=22): BW ↓7.6%, ALT ↓41%, AST ↓23%, liver spleen ratio ↑11%, HOMA ↓33%.

Data are mean ± standard errors usually rounded to the nearest full number. Arrows indicate the direction of change; changes reported were statistically significant p <0.05 unless noted otherwise. Sample size based reflects those in the final analysis. Abbreviations: ALT - alanine aminotransferase; AST - aspartate aminotransferase; BMI - body max index (kg/m^2); BW - bodyweight; d - day(s); CHO - carbohydrate; CT – computed tomography; d - day(s); E - energy; E-HC - euglycaemic-hyperinsulinaemic clamp; GRa - glucose rate of appearance; GR_d - glucose rate of disappearance; h - hour(s); HbA1c – glycated haemoglobin; HOMA - homeostasis model assessment; IHTAG - intrahepatic triacyglycerol concentration; IS - whole body insulin sensitivity; ITT – intention to treat analysis; LI, light intervention; MI, moderate intervention; MUFA - monounsaturated fatty acid; n - sample size of cohort in final statistical analysis; NAFLD - non-alcoholic fatty liver disease; NASH - non-alcoholic steatohepatitis; n/r, data not reported; n.s. – result not statistically significant; PP – per protocol analysis; PRO - protein; PUFA - polyunsaturated fatty acid; RCT – randomised controlled trial; RCTS, single arm of an RCT; RT – randomised trial with no control; RMR - resting metabolic rate; SAFA - saturated fatty acid; T2D, type 2 diabetes; UCT – uncontrolled trial; w – week(s).

2.3.2.4 Diet Combined with specific Physical Activity and/or Exercise Prescriptions

Five studies involving 306 participants (approximately 50% women, 10 controls) prescribed specific diets and aerobic exercise programs for 3-6 months [325,332-335]. Key study details are summarised in Table 2.4 The focus was predominantly on body weight reduction and maintenance with mean reductions of 4.2-10.6%. All studies reported reductions in direct measures of liver fat and/or liver enzymes; none used ^{1}H-MRS. Vilar-Gomez *et. al.* had the single largest cohort of biopsy assessed participants of the studies reviewed (n = 30) and reported significant ($p < 0.5$) reductions in inflammation, ballooning injury, and fibrosis, relative to baseline, following a six month intervention with a mean 10.6% weight reduction [332]. Ueno *et al.* reported significant reductions in steatosis, but reductions in other parameters were not statistically significant [333]. Relative mean reductions in IHTAG based on biopsy scores were 40-43% [332,333]. The four studies reporting glucose control/insulin sensitivity showed improvements [331-334].

Table 2.4: Interventions Combining Diet and Specific Physical Activity/Exercise Advice

Reference (Design)	Sample (M/F)	Clinical Group	Age (years)	BMI (kg/m^2)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(332) RCTS	30(16/14)	NASH	49±2	32±2	**Diet**: 500 kcal/d reduction (64% CHO, 22% fat, 14% PRO). **Physical Activity**: walking or jogging 40 min ≥5 d/wk.	26	Histopathology (NAS), ALT, AST, HOMA Daily E deficit of 370 kcal, macronutrient composition as prescribed (recall method n/r), PA related adherence was a score (questionnaire)	BW ↓10.6% NAS ↓51% , steatosis ↓40%, ballooning -58%, necroinflammation ↓65%, fibrosis ↓55%. ALT ↓41%, AST ↓48%, HOMA ↓35%
(333) NRCT	15 (8/7)	NAFLD	39±3.5	31±1	**LI**: 25 kcal/kg ideal BW/day (height (cm)-100x0.9) (50% CHO, 30% fat, 20% PRO) + exercise: 3000 steps/d increased by 500 every 3 days until 10000, then jogging for 20 min 2/d.	12	Histopathology ALT, AST, fasting glucose **LI** were inpatients for 4 wks then food provided.	**Relative to baseline**: BMI ↓9.7%. Steatosis ↓43%; n.s. change in portal or lobular infiltration, or fibrosis. ALT ↓67%, AST ↓59%, fasting glucose ↓14%.
	10 (5/5)	NAFLD	54±3	31±1	**CON**: (not randomised - inability to undertake inpatient protocol): no intervention.	12		N.s. changes in outcome measures. BMI ↑3.4%
(334) RCTS	22(8/14)	T2D	52±2	36±1	2002 American Diabetes Association position stand diet. Weekly dietitian visits. Fat ≤30% of E & negative E balance of ~ 500 kcal/d. Advice to increase moderate activity by 40-60 min/d.	26	CT (liver to spleen ratio). Glucose clamp & indirect calorimetry: GR_d, EGP, HbA_{1c} Diet (24 hr recall): -568 kcal/d, no change in macronutrients from baseline. PA n/r.	BW ↓9.8% Liver:spleen ratio ↑24%, GRd ↑35%, EGP ↓47%, HbA1c ↓12%.
(325) UCT	28(n/r)	High ALT	37(n/r)	29±1	Diet of 25 kcal/kg ideal BW ((height in cm - 100) x 0.9)) NCEP step 1 (60% CHO, 20% fat, 20% PRO & 200 mg cholesterol). PA: 45 min 6 d/wk at 60-70% estimated HR_{max}	12	ALT & AST Some sessions supervised. Only results for those completing ≥4 sessions/week included. Diet: n/r.	BMI ↓9.7% ALT ↓35%, AST ↓38%.

Reference (Design)	Sample (M/F)	Clinical Group	Age (years)	BMI (kg/m^2)	Intervention	Length (weeks)	Outcome Measures & Adherence	Results
(335) RCT	64(31/33)	T2D	56±1	32±0.5	American Diabetes Association: 23 kcal/kg/d (50-55% mixed GI CHO, 30% FAT, 15-20% PRO). PA: 30 min aerobic activity ≥3 d/wk	48	ALT Diet (FFQ): 2089 kcal (46% CHO, 37% fat, 19% PRO) & energy -39% vs. baseline (1348 kCal). PA: n/r	BW n/r ALT - 22%
	73(40/33)	T2D	57±1	31±0.5	Low GI Diet: 23 kcal/kg/d (50-55% low GI CHO, 30% FAT, 15-20% PRO). PA: 30 min aerobic activity ≥3 d/wk	48	ALT Diet (FFQ): 1987 kCal (45%CHO, 36% fat, 20% PRO) -37% vs. baseline (1189 kCal). PA: n/r	BW n/r ALT -18
	64(32/32)	T2D	55±1	31±0.5	Modified Mediterranean Diet: 23 kcal/kg/d (35% low GI CHO, 45% high MUFA FAT, 20% PRO). PA: 30 min aerobic activity ≥3 d/wk	48	ALT Diet (FFQ): 2226 kCal (42% CHO, 41% fat, 19% PRO) & energy -37% vs. baseline (1189 kCal). PA: n/r	BW n/r ALT -40

Data are mean ± standard errors usually rounded to the nearest whole number. Changes reported as percentages where possible. Changes reported were statistically significant $p < 0.05$ unless noted otherwise; Abbreviations: 2 h glucose - blood glucose concentration at 2 hours post oral glucose tolerance challenge; ALT - alanine aminotransferase; AST - aspartate aminotransferase; BMI - body max index (kg/m2); BW - bodyweight; d - day(s); CHO - carbohydrate; CT - computed tomography; d - day; E - energy; EGP - endogenous glucose production; E-HC - euglycaemic-hyperinsulinemic clamp; FFQ – food frequency questionnaire; GI - glycaemic index; GRa - glucose rate of appearance; GRd - glucose rate of disappearance; HISI - hepatic insulin sensitivity index; HOMA - homeostasis model assessment; IHTAG - intrahepatic triacylglycerol concentration; ITT - intention to treat analysis; MET - metabolically equivalent tasks; min - minute; mod - moderate; MUFA - monounsaturated fatty acid; NAFLD - non-alcoholic fatty liver disease; n/r - data not reported; n.s. - no statistically significant change; NAS - Non-Alcoholic Fatty Liver Disease Activity Score; NASH - non-alcoholic steatohepatitis; LBM - lean body mass; NRCT – non-randomised control group; OGTT - oral glucose tolerance test; PA - physical activity; PP - per protocol; PRO - protein; PUFA - polyunsaturated fatty acid; RCTS, single arm of an RCT; SAFA - saturated fatty acid; T2D, type 2 diabetes; wk - week.

2.3.2.5 Mediators of Liver Fat Reduction and Glucose Control/Insulin Sensitivity

Additional post hoc analyses to identify key determinants of liver fat reduction were done in three studies [8,242,327,331]. The percentage change in body weight was positively correlated with reductions in liver enzymes ($r = 0.5$), hepatic steatosis ($r = 0.6$), and overall NASH disease activity ($r = 0.5$) [327]. Cardiorespiratory fitness at baseline was found to be a better predictor of change in liver fat than baseline IHTAG, and visceral or total adipose tissue mass following a combined diet and physical activity intervention [8].

Improvements in overall NASH Score, steatosis, inflammation, ballooning injury, and fibrosis were significantly ($p < 0.05$) greater in those achieving weight reductions ≥7% of baseline body weight compared to those with smaller reductions [327]. When dividing their group (n = 15) into responders and non-responders based on total NASH Score, Huang *et al.* reported statistically significantly greater weight reduction (-6.6 vs. +1.8 kg) and questionnaire reported physical activity among responders compared with non-responders [331]. Further, increasing duration and frequency of physical activity was associated with increasing reductions in liver enzymes [242]. A similar relationship was observed between changes in cardiorespiratory fitness and liver enzymes, but only when results were compared relative to baseline rather than controls [242].

The only study included in this review to asses multiple time points over several weeks, showed a rapid – within 48 hours – positive effect of energy, particularly carbohydrate, restriction on IHTAG, hepatic insulin sensitivity, and hepatic glucose production [171]. An average of seven weeks later at a mean body weight reduction of approximately 7%, peripheral aspects of insulin action, specifically insulin mediated glucose uptake and insulin signalling, also improved.

2.4 Discussion

Lifestyle modification, with an emphasis on weight reduction, is the cornerstone of NAFLD management [13]. The findings of this systematic review support this recommendation. Lifestyle interventions focussing on weight reduction produced a consistent improvement in liver health, whether assessed by liver enzymes, imaging or biopsy. Weight reductions of 4-14% resulted in a relative reduction in IHTAG of 26 - 81%, with liver fat reductions tending to parallel body weight reduction. A limited number of exercise only interventions also identified the potential for more modest 10-20% reductions in IHTAG in the absence of weight change, and one study suggesting liver fat reduction can be rapid .

However, this review identified numerous study design and methodological issues with the current evidence base (*Section 2.3.1*). Others have noted that the few available randomised controlled trials of weight reduction in NAFLD carry a high risk of bias, and that poor reporting of adverse events leaves questions about the safety of these interventions incompletely addressed [336]. Such observations highlight the limitations of the current evidence base, but also the limitations of adhering strictly to best practice in the performance of systematic reviews, which requires that only studies in populations with the condition in question be considered.

Given the close association of NAFLD with the metabolic syndrome [2], and its high prevalence in the morbidly obese and those with type 2 diabetes [5,337], it seems prudent to consider the findings from interventions in these groups. Expanding the discussion beyond NAFLD research is particularly relevant in a review focusing on lifestyle modification, which is an acknowledged component of evidence-based treatment for the comorbidities and sequelae of NAFLD [2]. The evidence for the feasibility of weight and cardiometabolic risk reduction with lifestyle intervention in populations with a high probability of elevated IHTAG is substantial when considering findings from major diabetes prevention studies (DPS) and diabetes treatment trials including: the Diabetes Prevention Program [338]; the Finish DPS [339,340]; the China Da Qing DPS [341]; the European DPS [342,343]; meta-analyses of these and several smaller trials [344]; and the Look AHEAD trial [328,345-347].

The DPS and Look AHEAD trials employed multifactorial interventions encompassing dietary and physical activity advice often combined with various behaviour change techniques [339,342,343,348,349]. Interventions were often delivered in a multi-disciplinary setting with dieticians/nutritionists [339,342,349], exercise physiologists [349], physiotherapists [342], behavioural psychologists [349], and/or nurses providing dietary, physical activity, and behaviour change advice [349]. Seven of the interventions included in the present systematic review employed a comparable approaches [241,257,328-331]; one was a subset of the Look AHEAD cohort [329].

Initial mean weight reductions at one year in the DPS and the Look AHEAD cohorts have been 2%-10% [344,350]. Long-term followup of the DPS show significant reductions in the incidence of type 2 diabetes and improvements in cardiometabolic risk factors with intensive lifestyle intervention [351-353], but the effect on actual incidence of disease remains unclear [352,353]. The reviewed trials in NAFLD patients reported 4-14% weight reduction and similar reductions in cardiometabolic risk factors as the DPS; in addition they demonstrate reductions in IHTAG. However, studies in NAFLD cohorts lack long-term follow-up.

In NAFLD cohorts predictors of IHTAG reduction were: percentage weight reduction [327]; cardiorespiratory fitness at baseline [8]; and physical activity [241,331]. The key determinants of improvement in glucose control in DPS were: weight reduction [342,344]; increased cardiorespiratory fitness [343]; and increased physical activity [341,342,354,355]. Whereas the strongest predictors of weight reduction were self-reported physical activity and treatment attendance [356]. Weight maintenance following reduction in NAFLD cohorts has not been assessed. However, lifestyle interventions in closely related patient groups indicate better weight maintenance was related to: larger initial reductions in weight [357-359]; sustained weight reduction during the active intervention period [359]; more physical activity [358], and greater attendance of lifestyle counselling sessions [358].

Translation of the DPS programmes has proven feasible and modestly successful, but so far only on a small scale [360,361]. In the absence of specific

lifestyle programmes for NAFLD, more generic weight reduction and physical activity promotion schemes may be appropriate. In the UK, exercise on referral schemes allow primary and secondary care to refer patients to regional initiatives promoting exercise and weight reduction [362-364], and the Counterweight programme offers evidence-based weight reduction counselling designed for delivery in the primary care setting [365,366].

Dietary advice in multi-factorial lifestyle interventions has tended to focus on overall dietary energy reduction by fat restriction [241,257,328-331,339,342,343,348,349]. Conversely, diet only interventions in the reviewed NAFLD cohorts ranged from modest daily energy reduction to very-low calorie approaches, and from high-carbohydrate/low-fat to high-fat/low-carbohydrate diets.

Low- and very low-calorie diets led to the significant weight, IHTAG, and liver enzyme reductions [171,313-315,318,319,322-324,367]. A recent study of very-low calorie consumption in type 2 diabetes similarly reported a large and rapid, significant within one week, reduction in IHTAG associated with the normalisation of several metabolic parameters over eight weeks [172]. Unfortunately, long-term followup was absent from all these studies. However, meta-analysis of randomised controlled trials, not specifically in NAFLD patients, reported comparable long-term (1-5 years) 5-6% bodyweight reduction from low and very-low calorie diets [368]. Further, a review of several weight reduction trials showed that the magnitude of initial weight reduction, irrespective of the approach used, is a good predictor of long-term maintenance [357]. Thus low-calorie diets followed by a programme to support weight maintenance should be considered an option in clinical care.

The composition of the diets affected how rapidly they reduced IHTAG. Compared to low-fat/high-carbohydrate diets severely restricting carbohydrates to <90 g/day showed greater reductions in IHTAG over 48 hours [171], and 14 days [173]. Indeed a 1000 kcal/day energy deficit with 10% of energy coming from carbohydrates and 75% from fat, resulted in a ~30% reduction in IHTAG and marked improvements in glucose control and insulin sensitivity within 48 hours, and with no significant further improvements after a 7% weight reduction [171]. However, over periods of 11 weeks or longer there was no difference with

respect to IHTAG reduction between diets either low or high in carbohydrates [171,319]. The utility of low-carbohydrate, sometimes ketogenic diets, was also shown in single arm studies [320,321].

Interestingly, a study forming part of the present systematic review most elegantly demonstrates that low-carbohydrate intake has a different physiological effect than weight reduction *per se.* Hepatic insulin sensitivity was improved by ~150% after 48 hours of energy and carbohydrate restriction with no further change after a 7% weight and 30% IHTAG reduction [171]. Whereas energy reduction with fat restriction resulted in a much more modest 50% improvement in hepatic insulin sensitivity within 48 hours with no further improvement following a 7% weight reduction despite further reductions in IHTAG. Multiple mechanisms would explain this rapid reduction in IHTAG with low carbohydrate intake: 1) the likely shift from TAG synthesis to ketone production and export [369]; reduction of *de novo* lipogenesis [101,370,371], a major contributor to IHTAG in NAFLD [97]; and greater reliance of the liver on endogenous and exogenous lipid as a fuel source (*Section 1.3* & *Section 1.7.4*.)

Concern about the safety of low-carbohydrate and therefore higher fat and/or protein diets is often voiced. Assessment of the available literature comes with the key caveats: 1 many trials reporting their use did not achieve sufficient adherence to the intended diet to answer questions of safety or effectiveness [372-380]; and 2) the studies reporting elevated cardiometabolic risk did not include any groups that were truly low-carbohydrate [381-383].

When considering only controlled studies in which published intake data indicates close adherence to low-carbohydrate intake of ≤100 g/day, and for the period in which this intake was maintained, such diets were comparable or superior to low-fat high-carbohydrate diets in terms of: weight reduction [372,377,384-390]; blood lipid profile [372,377,384,386-393]; fasting glucose [372,377,384,385,392,394]; fasting insulin [372,377,389,392,394]; HOMA-IR [388,392,394]; 24-hour glucose response [385]; 24-hour insulin response [385]; HbA_{1c} [388,389,395]; and postprandial lipaemic response [392].

Anthropological evidence of modern and paleo/neolithic hunter gatherer societies support a long history of diets relatively low (approximately 35%) in energy from plant material and therefore carbohydrates, and high (approximately 65%) in energy from animals and therefore protein and fat [396-398]. Within these hunter gatherer populations such relatively animal protein and fat rich diets were not associated with indicators of elevated cardiometabolic risk [397]. The few interventions seeking to mimic such diets supports their positive effects on: insulin sensitivity [399]; glucose control [400], blood lipids[399,400]; blood pressure [399,400]; body weight [400]; and satiety [401]. The studies to date have been small with no long-term followup published. They are compelling because one study intentionally maintained weight [399], one used the Consensus Mediterranean diet in their comparison group [401,402], and one was designed as a cross-over with the comparison diet consistent with standard recommendations for management of type 2 diabetes [400].

There is considerable evidence to support a broad range of macronutrient distributions, which if consumed in an amount that induces weight reduction, are consistent with improved cardiometabolic risk profiles and IHTAG reduction. Specific restriction of carbohydrates is particularly appropriate in NAFLD and those with impaired insulin sensitivity. However, ultimately the decision of what dietary approach to recommend should be pragmatic and consistent with what each patient feels willing and able to do. Any diet should also be consistent with established recommended protein [403], essential fat [404], and micronutrient intakes [405]. Appropriate support to achieve prolonged adherence to the chosen dietary approach is likely to be more important than its macronutrient composition. This support should ideally come in the form of theory-based behaviour change programmes. The most appropriate programme for NAFLD remains to be determined. In the interim, use of locally available schemes is appropriate.

It is evident from multifactorial lifestyle interventions that physical activity is associated with treatment success in terms of weight maintenance and long-term disease prevention [351-353]. The exercise only arm of the Da Qing DPS had the greatest reduction in six year incidence of type 2 diabetes [341]. As noted in *Section 1.7.5*, cross-sectional and retrospective data indicate that total physical

activity, vigorous physical activity, cardiorespiratory fitness, and resistance training are all inversely associated with the risk of NAFLD and its severity. Notably, these findings were largely independent of BMI, but unfortunately body composition was not assessed more directly.

Prospective investigations support a protective role of physical activity with respect to NALFD. In a diet and exercise intervention, baseline cardiorespiratory fitness predicted IHTAG reduction in both NAFLD and non-NAFLD cohorts, and remission in the NAFLD cohort, independent of change in total physical activity [8]. However, increased total physical activity was also associated with IHTAG reduction. The five exercise only interventions included in this review consistently reported modest reductions in IHTAG independent of weight reduction. This was despite the range of frequency and exercise types employed. Increased cardiorespiratory fitness was positively related to reductions in IHTAG [313], and liver enzymes [328]. The effect of exercise on glucose control/insulin sensitivity was not so conclusive. This was in part due to only three studies reporting relevant markers, and one study finding no change in HOMA-IR following a four week intervention. However, meta-analyses indicate that continuous aerobic exercise has the potential to produce clinically meaningful improvements in: glucose control [274], blood pressure, and serum triacylglycerol in type 2 diabetes [275]; visceral fat [406], markers of inflammation [407]; HDL-cholesterol [277,278]; and blood pressure in those with hypertension [276]. Similar analysis of interventions employing resistance exercise have shown benefits including: improved glucose control in type 2 diabetes [274,408]; reductions in hypercholesterolaemia and hypertriacylglycerolaemia [409,410]; and reductions in body fat [410].

Collectively observational and intervention data support the utility of a broad range of physical activity options in the prevention and treatment of NAFLD; as both an adjunct too and independent of weight reduction. Exercise of higher intensity and exercise of an anaerobic nature, e.g. resistance training, may be particularly effective. However, simply increasing time spent active is likely to be beneficial and should be strongly encouraged, especially in those reluctant to do structured vigorous exercise, but also as an adjunct in those who are willing to exercise. As with dietary and broader lifestyle interventions, long-term

adherence is likely to be improved by employing formal behavioural modification techniques such as those employed in multifactorial lifestyle interventions.

2.4.1 Critique of NAFLD Studies and Recommendations

Overall, the studies on lifestyle intervention in patients in NAFLD have several limitations. Most notably: considerable heterogeneity in the populations studied; limited characterisation of risk factors prevalent in volunteers, adherence to specific aspects of the interventions or recording/reporting of lifestyle changes beyond those advocated by the interventions; and too little published detail on interventions to allow accurate replication in research or clinical practice. Future studies should focus on:

Improved reporting by:

- transparent and comprehensive descriptions of diagnostic markers of NAFLD including inclusion and exclusion criteria;
- thorough assessment of potential contributors to NAFLD, such as nutrient excess or deficiency where these are likely to be prevalent in the study population and influenced by the intervention; and
- publication of supplementary material describing complex lifestyle interventions in sufficient detail for these to be replicated or adapted to clinical practice.

Selection of study methods:

- quantitative assessments of hepatic steatosis;
- quantitative assessment of NASH activity via biopsy or closely correlated activity scores such as the NAS Activity Score;
- objective physical activity monitoring methods;
- validated dietary assessment methods;
- comprehensive exclusion of specific causes of steatosis, e.g. nutrient excess or deficiency; and
- validated methods to quantitatively assess alcohol consumption

Research focus:

- characterisation of behavioural change techniques most effective in NAFLD cohorts;
- definition of the dose response relationship for physical activity/exercise intervention and IHTAG reduction;
- the effect of dietary macronutrient composition on IHTAG, especially carbohydrate restriction and higher protein intakes;
- a graduated progression from Phase II to Phase III trials; and
- a focus on how both diet and physical activity/exercise can be used to produce sustained benefit in NAFLD.

2.5 Conclusions

This systematic review of lifestyle interventions identified consistent evidence that lifestyle interventions designed to reduce energy intake and/or increase physical activity to induce weight reduction can reduce IHTAG and improve insulin sensitivity in patients with NAFLD. A more limited data set indicates a trend for reductions in necroinflammation following weight reduction focused intervention. The effect on fibrosis is least consistent across studies. Degree of weight reduction is positively correlated with improvements, as is cardiorespiratory fitness.

However, contrary to the current emphasis on weight reduction, studies of carbohydrate restriction indicate this to be effective at reducing IHTAG and improving glucose metabolism even with very modest weight reduction. Notably, exercise only interventions, although underrepresented in the literature, appear to be a therapeutic option that is effective in the absence of dietary advice and independent of weight change.

When broadening the evidence base beyond clinically defined NAFLD or NASH to include groups with a probable high prevalence of NAFLD, the evidence base becomes stronger. Energy restriction resulting in weight reduction, irrespective of macronutrient composition, is likely to reduce IHTAG. However, sufficient protein and micronutrient consumption should be ensured.

Exercise, with or without concomitant weight reduction is likely to result in modest reductions in IHTAG and a substantial reductions in cardiometabolic risk. However, the NAFLD specific evidence base in terms of appropriate mode and dose of exercise remains in considerable need of expansion.

3.0 Review of High-Intensity Intermittent Training

3.1 Introduction

Exercise is a recommended component of lifestyle therapy for non-alcoholic fatty liver disease (NAFLD) [14,311]. Exercise is also recommended for the treatment and prevention of several common NAFLD comorbidities and sequelae, including obesity [411,412], impaired glucose tolerance and type 2 diabetes [413,414], and cardiovascular diseases [415]. The benefits of exercise for these conditions are recognised in related UK treatment and prevention guidelines [416]. Meta-analyses support these guidelines by indicating that continuous moderate intensity exercise (CMIE) has the potential to produce clinically meaningful improvements in glucose control [274], blood pressure, and serum triacylglycerol in type 2 diabetes [275], and blood pressure in those with hypertension [276], as well as reductions in visceral fat [406], markers of inflammation [407] and HDL-cholesterol in mixed populations [277,278]. Similar analyses of interventions employing resistance exercise have shown improved glucose control in type 2 diabetes [274,408], reductions in hypercholesterolaemia and hypertriacylglycerolaemia [409,410], and body fat [410] in mixed populations. As well as physical benefits, exercise has been shown to improve depression secondary to chronic illness [417]. Exercise is also a valuable adjunct to energy restriction when weight, particularly body fat reduction, is the goal, and is a key of component in weight maintenance [412,418,419].

However, only five published studies have looked at exercise only interventions in patients with clinically defined NAFLD [243-245,325,326]: one assessed steatosis by liver histology following resistance exercise [326], one relied on aminotransferase changes to measure change following CMIE [325], and the remaining three employed proton-magnetic resonance spectroscopy to quantify liver fat following CMIE [244,245] and resistance exercise [243], respectively. All studies reported modest relative reductions in liver fat of 10-21% independent of weight reduction (see *Chapter 2*).

High-intensity intermittent training (HIIT), often referred to as *interval training*, is a particularly time efficient mode of exercise, which has shown promise in

populations with metabolic syndrome and those with type 2 diabetes [420-425], but has not been formally assessed in populations with confirmed NAFLD. Incorporation of HIIT into lifestyle therapies for NAFLD patients would seem consistent with recent findings indicating regular vigorous physical activity is associated with a reduced risk of developing NASH [240], and a recent meta-analysis encompassing data from over 1.3 million volunteers enrolled in cohort studies reporting risk reduction of all cause mortality was greatest in response to vigorous activity on a per unit time basis [426]. Further, intensity of continuous aerobic exercise is associated with improvements in insulin sensitivity [427-429], reductions in visceral fat [430-432], and blood pressure reductions [433]. However, prolonged periods of exercising at high intensity are unsuitable for many patients with NAFLD, many of whom are generally sedentary [232,233,236,434], have reduced exercise tolerance [181], and lack confidence in performing exercise [435]. HIIT represents an approach to exercise that allows accumulation of time exercising at high intensity without demanding this to be maintained for prolonged periods.

This chapter will summarise the rationale for testing this mode of exercise in a group with NAFLD by reviewing research conducted in non-athletic populations with emphasis on findings in those with common comorbidities of NAFLD, and research looking specifically at relevant physiological changes induced by HIIT.

The following sections assess the likely suitability of HIIT for the treatment of NAFLD by:

- Defining the nature of HIIT;
- Establishing the safety and tolerability profile of HIIT; and
- Reviewing the known physiological changes likely to be of relevance in the context of NAFLD, specifically with respect to:
    - Cardiorespiratory and cardiovascular fitness and health;
    - Body weight and composition; and
    - Fasting and postprandial metabolism

3.2 Methods

3.2.1 Selection Criteria and Search Strategy

The review is restricted to published prospective studies reporting the effects of HIIT, as defined in *Section 3.2.3* below, in healthy and clinical populations, but not those engaged in a formal training programme for competitive sport. Clinical populations considered most relevant were those with one or more components of the metabolic syndrome present (*Section 1.8.1*). Research in those with chronic obstructive pulmonary disease, cystic fibrosis, spinal injury, intermittent claudication, or other rarer conditions potentially requiring very specific adjustment of exercise prescription were excluded [436]. Emphasis is placed on studies examining the safety and tolerability of HIIT in clinical populations, and those reporting its effects on cardiorespiratory fitness, body composition, and recognised cardiometabolic risk factors.

The online version of Medline (Pubmed) was searched via Papers 2.0 (Mekentosj, Aalsmeer, The Netherlands) using the search terms: *high intensity intermittent training*, *sprint interval training*, and *interval training* in the title or abstract of papers. The reference lists of papers identified, including reviews, were also searched for potentially relevant papers. This was not a formal systematic review and only English language papers available through the libraries of Newcastle University were included.

3.2.2 Data Extraction

Data on study volunteer demographics, intervention protocols, and outcomes were extracted into tables. Outcome data were converted to give percentage change for all parameters of interest.

3.2.3 Types and Definitions of High Intensity Intermittent Training

No single universally adopted definition of HIIT exists. Published protocols generally
involve multiple intervals of high-intensity activity, commonly walking/running or cycling, lasting 1-4 minutes and interspersed with active or passive recovery

periods of similar length (see *Tables 3.1-3.5* for examples). Intensity is commonly defined in terms of the percentage of peak volume of oxygen consumed (VO_{2peak} or VO_{2max}), peak heart rate (HR_{peak} or HR_{max}), or self-reported rating of perceived exertion. High intensity has generally been defined as 80-95% of VO_{2peak}/VO_{2max}, HR_{peak}/HR_{max}, or a perceived rate of exertion ≥16 on a scale of 6-20 (see Appendix 1). Passive recovery involves rest, whereas active recovery involves light to moderate intensity activity corresponding to ≤60% VO_{2peak}/VO_{2max} or HR_{peak}/HR_{max} (see *Tables 3.1-3.5* for examples). Peak or maximum intensity are obtained using graded maximal exercise tests.

However, actual VO_2 was not generally assessed during exercise outside of graded exercise testing, instead the resistance or speed of each interval are matched to correspond with the point during a graded maximal exercise test at which the desired intensity was reached. Sprint interval training (SIT) is a subset of HIIT in which intervals are less than a minute long and often exceed maximum aerobic capacity by requiring volunteers give an ‘all out’ effort for the prescribed time. Both HIIT and SIT use multiple intervals to accumulate total time at intensities the trainee could not maintain for prolonged periods.

3.3 Results

3.3.1 Safety and Tolerability of High-Intensity Intermittent and Sprint Interval Training

The high intensity nature and therefore cardiac demand of HIIT and SIT exercise raises the question of their safety in clinical populations at increased risk of or with coronary heart disease. Studies assessing the safety, tolerability, and/or acute cardiovascular response to HIIT & SIT are summarised in *Table 3.1*. The largest assessment of safety for HIIT was a clinical audit involving 4846 coronary heart disease patients enrolled in Norwegian cardiac rehabilitation programs [437]. This survey identified only two non-fatal cardiac arrests in 46,364 exercise hours for the HIIT protocol, whereas there was one fatal cardiac arrest in 129,456 exercise hours of CMIE. Although this translates into a higher rate for HIIT, this was a function of time as the HIIT protocol only required 36% of the exercise time required by the CMIE protocol. Due to the low frequency of events, the study was underpowered in terms of being able to detect a difference in risk for HIIT vs. CMIE, but indicated the risk for either exercise approach is low. Intervention trials with much smaller cohorts in populations with chronic heart failure [438-446], recent stroke survivors with hemiparesis [447], and patients with coronary heart disease have reported no serious adverse events [448].

3.3.1.1 Acute Cardiovascular Response

Studies examining the acute cardiovascular response to exercise are summarised in *Table 3.1*. In patients with coronary heart disease undergoing various ECG and blood pressure monitored HIIT and SIT (≤100% of peak aerobic capacity, not supramaximal as is common with SIT) protocols, no contraindications to continuing the protocols were observed and undesirable changes such as ST-segment depression recovered during between-interval recovery periods [448,449]. In patients with chronic heart failure, cardiac stress as assessed by rate pressure product (heart rate x systolic blood pressure) stayed within acceptable values [450]. In patients with metabolic syndrome, flow mediated dilation – a measure of arterial response to shear stress – increased following HIIT [423]. In patients with pharmacologically treated hypertension, 24-hour and

daytime systolic blood pressure were reduced following HIIT [451]. Further, the blood pressure of healthy individuals performing a common SIT protocol was not excessively elevated, and reductions in central and peripheral pulse-wave velocity – a measure of arterial stiffness – observed immediately following exercise returned to baseline within 20 minutes post-exercise [452]. Supine resting blood pressure was reduced by HIIT in active volunteers for at least an hour post-exercise, but returned to baseline by 24 hours [453]. Likewise, in a healthy cohort, R-R interval – a measure of heart rate variability and sympathetic/parasympathetic balance – was decreased following HIIT relative to CMIE but recovered within 48 hours [453,454], whereas pulse wave velocity was comparable between HIIT and CMIE [454]. Thus the acute cardiovascular response to HIIT is within acceptable parameters even in patients with diagnosed stable cardiovascular disease.

Three studies comparing SIT and HIIT protocols of different interval lengths reported that longer intervals result in greater periods spent at higher intensities in terms of VO_2, and therefore represent a greater physiological stimulus [455-457]. A fourth study, notably in men with chronic heart failure, observed marked reductions in total exercise time when passive between interval recovery periods were replaced with active ones [444]. However, total time at intensities greater than 100%, 95%, and 90% of VO_{2peak} were maintained even as total exercise time was reduced.

3.3.1.2 Tolerability

Studies seeking to assess the participant tolerance of HIIT and SIT protocols are summarised in *Table 3.1.* Interval power output tends to decline from interval-to-interval [452], whereas feelings of leg fatigue and breathlessness tend to increase [450]. Volunteer reported post session ratings of perceived exertion ranged from 14-18 on scale going from 6-20 points [448,449]. Despite this relatively high perceived exertion volunteers rate HIIT and SIT preferable to CMIE protocols at similar total workloads [449,458,459]. A comparison of protocols in a cohort with chronic heart failure reported a greater perception of exertion with active vs. passive recovery periods; this was accompanied by markedly reduced time to exhaustion [444]. Tolerability was maintained in cohorts on heart

rate limiting β-blockers [444,448,450]. Thus intermittent exercise protocols with intervals at maximum aerobic capacity are tolerated in a broad range of clinical populations. However, SIT protocols with efforts exceeding maximal aerobic capacity remain largely untested in clinical populations with evidence of cardiovascular disease.

3.3.1.3 Alternative Measures of Intensity

There are inherent difficulties with setting intensity using objective laboratory measures, most notably the need for equipment to measure these parameters, and/or to set exact work rates/resistance. There is also the need for the exerciser to understand what is required if exercising unsupervised or be supervised. Such requirements are potentially difficult to meet outside a supervised research setting. Recent work in trained runners has examined the suitability of using rating of perceived exertion and a perceived readiness scale in self pacing HIIT sessions [460]. Use of rating of perceived exertion resulted in a better time to complete multiple 1000 m runs with self-pacing than pacing based on heart rate recovery, and a shorter rest times than when using a set 1:1 rest-to-work ratio. Rating of perceived exertion was also successfully employed in active young women to produce comparable elevations in VO_{2peak} to those achieved using an objective intensity prescription [461].

3.3.1.4 Risk Assessment Summary

Although not definitive, these data are reassuring as they suggest a low cardiac risk of HIIT protocols even in particularly vulnerable populations. Nonetheless, the remaining uncertainty justifies screening of at risk populations, preferably by ECG-monitored graded maximal exercise test to help identified previously undiagnosed cardiac abnormalities. Until there is more data on the safety and tolerability of supramaximal effort SIT protocols in higher risk populations, these should not be utilised outside of supervised and appropriately monitored situations.

Table 3.1: Safety and Tolerability of High-Intensity Intermittent Training and Sprint Interval Training

Reference	Population	Protocol	Tolerability	Safety
437	3392 men & 1454 women, cardiac rehabilitation patients, 58±n.r. years, BMI n.r., VO_2peak n.r.	**HIIT:** walking at 80-90% VO_2peak for 4x4 min with 3 min active recovery at 50-60% VO_2peak for a total of 46364 person hours		2 non-fatal cardiac arrests
		CME: walking at 60-70% VO_2peak for ~ 60 min for a total of 175820 person hours		1 fatal cardiac arrest
448	17 men & 2 women, coronary heart disease, 65±8 years, BMI 28±4, VO_{2peak} 27±7	**SIT1**: 15 s at 100% WR_{max} & 15 s passive recovery until volitional exhaustion or a maximum of 35 min	RPE post session 15; time to exhaustion 29 min; time above 90% & 95% VO_{2max} 7.2 min & 4.6 min, respectively	n.s. arrhythmias or abnormal BP response (parameters not defined), mild ischaemia (ST depression 1.1 mm, n=3)
		SIT2: 15 s at 100% WR_{max} & 15 s active recovery 50% WR_{max} until volitional exhaustion or a maximum of 35 min	RPE post session 17, time to exhaustion 12 min, time above 90% & 95% VO_{2max} 7.4 min & 5.6 min, respectively	n.s. arrhythmias, mild ischaemia (ST depression 1.1 mm, n=3), no abnormal BP response
		HIIT1: 1 min at 100% WR_{max} & 1 min passive recovery until volitional exhaustion or a maximum of 35 min	RPE post session 17, time to exhaustion 25 min, time above 90% & 95% VO_{2max} 5.5 min & 3.7 min, respectively	n.s. arrhythmias, mild ischaemia (ST depression 1.6 mm, n=3), vagal reaction (n=2), no abnormal BP response
		HIIT2: 1 min at 100% WR_{max} & 1 min active recovery 50% WR_{max} until volitional exhaustion or a maximum of 35 min	RPE post session 18, time to exhaustion 14 min, time above 90% & 95% VO_{2max} 7.2 min & 5.1 min, respectively	n.s. arrhythmias, mild ischaemia (ST depression 1.5 mm, n=3), no abnormal BP response

Reference	Population	Protocol	Tolerability	Safety
449	19 men & 1 women, coronary heart disease, 62±11 years, 27±4, VO_{2peak} 28±9	**SIT**: 2 x 10 min bouts of 15 s cycle sprints at peak power output (corresponding to VO_{2peak}) with 15 s passive rest & 4 min passive rest between bouts	RPE post session 14; preferred over CME	n.s. arrhythmias or abnormal BP response (parameters not defined); serum cTnT <0.04 mg/L at all times (baseline, 20 min and 24 hr post exercise)
		CME: 70% of peak power output (corresponding to VO_{2peak}); duration set to be isocaloric with SIT session (mean duration of 28.7 minutes)	RPE post session 16	n.s. arrhythmias or abnormal BP response; serum cTnT <0.04 mg/L at all times (baseline, 20 min and 24 hr post exercise)
454	11 men, healthy, 23±2 years, BMI 22±n.r., VO_{2peak} n.r.	**HIIT:** 1 min interval at >85% HR_{max} with 3 min active recovery at ~ 65% HR_{max} x 6	Mean Q 15.9 L/min, max Q 21.7 L/min both greater than CME	R-R interval ↓28% greater than CME Arterial stiffness (assessed by pulse wave velocity) n.s.
		CME: workload adjusted to maintain the average HR of HIIT for 30 minutes	Mean Q 13.9 L/min, max Q 14.8 L/min	R-R interval ↓17% Arterial stiffness (assessed by pulse wave velocity) n.s.
450	16 men, chronic heart failure, 54±9 years, BMI 25±n.r., VO_{2max} 15±1	**SIT1:** 30 s cycle intervals at 50% W_{max} with 60 s active recovery against 15 W resistance x 6	Δ between early & later intervals: leg fatigue ↑6%; dyspnoea ↑6%; HR, VO_2 & VCO_2, adrenaline & noradrenaline n.s.	cardiac stress (RPP) at last interval 84 (<than at 75% VO_{2peak} during graded maximal exercise test)
		SIT2: 15 s cycle intervals at 70% W_{max} with 60 s active recovery against 15 W resistance x 9 matched for work with Trial 1	Δ between early & later intervals: leg fatigue ↑9%; dyspnoea ↑14%; adrenaline ↑42%; noradrenaline ↑41% HR, VO_2 & VCO_2 n.s.	cardiac stress (RPP) at last interval 88 (<than at 75% VO_{2peak} during graded maximal exercise test)
		SIT3: 10 s cycle intervals at 80% W_{max} with 60 s active recovery against 15 W resistance x 11 matched for work with Trial 1	Δ between early & later intervals: leg fatigue ↑10%; dyspnoea ↑8%; HR ↑4%; SBP ↑8%; VO_2 & VCO_2, adrenaline & noradrenaline n.s.	cardiac stress (RPP) at last interval 86 (<than at 75% VO_{2peak} during graded maximal exercise test)

Reference	Population	Protocol	Tolerability	Safety
457	9 men, active, 20±1 years, 24±n.r., VO_{2peak} 46±6	**SIT single: 1** x 30 s cycle sprints against 7.5% BW resistance; measurements done for 60 min post exercise	specific aspects n.r.; all volunteers completed the protocol	SBP ~ 155 mmHg & DPB ~ 74 mmHg 2 min post exercise; HR remained elevated over 60 min; BP returned to baseline by 15 min; central PWV ↑ n.r. returning to baseline by 20 min; peripheral PWV ↓ n.r. returning to baseline
		SIT multiple: 4 x 30 s cycle sprints against 7.5% BW resistance with 4.5 min active recovery (40 W at >50 rpm) between sprints; measurements done for 60 min post exercise	relative to first bout mean power decreased by 15%, 25%, 28% in subsequent three bouts, respectively	SBP ~ 155 mmHg & DPB ~ 74 mmHg 2 min post exercise; HR remained elevated over 60 min; BP returned to baseline by 15 min; central PWV ↑ n.r. returning to baseline by 20 min; peripheral PWV ↓ n.r. returning to baseline
451	8 men & 18 women, pharmacologically treated hypertension, 44±9 years, BMI 28±5, VO_{2peak} n.r.	**HIIT**: 40 min alternating 2 min 50% HRR & 1 min 80% HRR.	24 hr SBP ↓2.2% & night time SBP ↓2.9%; 24 hr, daytime, & nighttime DBP n.s.	
	16 men & 10 women, pharmacologically treated hypertension, 48±7 years, BMI 27±4, VO_{2peak} n.r.	**CME**: 40 min cycling at 60% HRR	24 hr SBP ↓2.1%, 24 hr DBP ↓2.9%, nighttime SBP ↓4.1%, & nighttime DBP ↓6.2%	
453	10 men, active, 25±2 years, BMI ±n.r., VO_{2peak} n.r.	**HIIT**: 1 min cycling at W_{max} x 9 bouts with 4 min active recovery periods at VT	5, 10, 15, 60 min, & 24 hr post exercise relative to baseline: RRI ↓35%, SBP ↓5.1% & DBP n.s.; RRI ↓32%, SBP ↓3.7% & DBP n.s.; RRI ↓29%, SBP ↓4.1%& DBP ↓6.2%; RRI, SBP & DBP n.s., respectively N.s. differences vs. CME	
		CME: cycling at VT matched with HIIT for total work performed	5, 10, 15, 60 min, & 24 hr post exercise relative to baseline: RRI ↓33%, SBP ↓6.3% & DBP n.s.; RRI 29%, SBP ↓7.5% & DBP n.s.; RRI 25%, SBP ↓9.5% & DBP n.s.; RRI ↓9.4%, SBP ↓7.8% & DBP ↓10%; RRI, SBP & DBP n.s., respectively	

Reference	Population	Protocol	Tolerability	Safety
456	14 men & 3 women, moderately trained, 22±4 years, BMI n.r. (BW 74±11 kg), VO2peak 57±	**HIIT1**: 10 x 1 min intervals at 100% VO_{2peak} with 1 min active recovery periods at 50% VO_{2peak} x single session	Mean VO_2 was 75.7% VO_{2peak}	
		HIIT2: 5 x 2 min intervals at 100% VO_{2peak} with 1 min active recovery periods at 50% VO_{2peak} x single session	Mean VO_2 was 78.6% VO_{2peak} (greater than HIIT1 & CME)	
		CME: 20 min cycling at 75% VO_{2peak} x single session	Mean VO_2 was 71.6% VO_{2peak}	
423	4 men & 7 women, metabolic syndrome, 55±13 years, BMI 30±2, VO_{2max} 34±3	**HIIT**: 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max}	FMD ↑120% for 48 hrs & above baseline for 72 hr, FG ↓~15% below baseline for 72 hr vs CON	
	4 men & 4 women, metabolic syndrome, 52±11 years, BMI 29±2, VO_{2max} 36±3	**CME**: 47 min at 70% HR_{max} (matched for energy expenditure with HIIT)	FMD ↑60% acutely & above baseline for 24 hr, FG ↓~15% below baseline for 24 hr	
	5 men & 4 women, metabolic syndrome, 50±9 years, BMI 32±1, VO_{2max} 32±3	**CON**: resting	FMD & FG n.s.	

Reference	Population	Protocol	Tolerability	Safety
444	22 men, chronic heart failure, 60±9, BMI 30±5, VO_{2peak} 17±5	**SIT1**: 30 s intervals at peak power (100% VO_{2peak}) with 30 s passive recovery until exhaustion or 30 min	Total exercise time 27.5 min (longer than SIT2 & HIIT2) Time % of VO_{2peak}: >100% 109 s ., >95% 190 s , >90% 316 s (all shorter than other protocols) Association of VO2peak with total exercise time *r*= 0.48 RPE15	
		SIT2: 30 s intervals at peak power (100% VO_{2peak}) with 30 s active recovery at 50% peak power until exhaustion or 30 min	Total exercise time 16.4 min Time % of VO_{2peak}: >100% 194 s, >95% 332 s, >90% 458 (all n.s. to other protocols except SIT1) Association of VO2peak with total exercise time *r*= 0.61 RPE18	
		HIIT1: 90 s intervals at peak power (100% VO_{2peak}) with 90 s passive recovery until exhaustion or 30 min	Total exercise time 26.2 min (longer than SIT2 & HIIT2) Time % of VO_{2peak}: >100% 132 s, >95% 228 s, >90% 344 s (all n.s. to other protocols except SIT1) Association of VO2peak with total exercise time n.s. RPE 16	
		HIIT2: 90 s intervals at peak power (100% VO_{2peak}) 90 s recovery at 50% peak power until exhaustion or 30 min	Total exercise time 16.0 min Time % of VO_{2peak}: >100% 184 s, >95% 324 s, >90% 488 (all n.s. to other protocols except SIT1) Association of VO2peak with total exercise time *r*= 0.54 RPE 17	

Study population demographics (sample size by sex, health or activity description, body mass index in kg/m2, and either VO_{2peak} or VO_{2max} in mL/kg/min as reported by study authors in are provided as means ± standard deviation, where reported, rounded to nearest whole number. Sample sizes are based on those included in the final analysis. Where data was reported in graph form it may not have feasible to accurately calculate percentage change so ~ is used to indicate this. The protocol column contains the core exercise but does not describe warm up and cool down protocols, which consisted predominantly of 5-10 minutes periods of light-to-moderated intensity activity. **Abbreviations**: BP, blood pressure; bpm, beats per minute; CME, continuous moderate-intensity exercise; cTnT, cardiac troponin; DBP, diastolic blood pressure; HIIT, high intensity intermittent training; HR, heart rate; n.r., data not reported or not reported in numerical form (e.g. only shown as a graph); n.s., no statistically significant change/difference; Q, cardiac output; rpm, revolutions per minute; RPE, rate of perceived exertion; RPP, rate pressure product (HR x SBP); SBP, systolic blood pressure; SIT, sprint interval training; ST, the ST segment of an electrocardiogram; VO_2, volume of oxygen consumption; VT, ventilatory threshold; WR, work rate.

3.3.2 Cardiorespiratory Effects of High Intensity Intermittent and Sprint Interval Training

Summaries of studies examining the cardiorespiratory and cardiovascular adaptations to HIIT and SIT are provided in *Table 3.2*. Numerous studies have shown marked improvements in cardiorespiratory fitness (fitness) and work capacity following HIIT and SIT (n=703) [421,422,424,438,445-447,451,457,461-501]. Changes ranged from 0-35% improvements in VO_{2peak} (unadjusted median 15%); 6.5-33% improvements in peak work rate, and 23-56% improvements in time to exhaustion.

Improvements in fitness were achieved in as few as six sessions of HIIT or SIT. Although not entirely consistent across studies, durations above six weeks and 18 sessions resulted in greater improvements in fitness and work capacity than shorter intervention durations. The three studies that measured changes in fitness at intermediate time points indicated improvements were rapid and increased over at least eight weeks [482,497,501]. Thirty seven (79%) of studies used a training frequency of three sessions per week, two used two or fewer, and seven used four or more sessions per week. No study compared multiple training frequencies. Given the heterogeneity of intervention designs, study lengths, and cohorts no clear dose response can be established. However, no clear advantage of frequencies exceeding thrice weekly is evident, and as few as 6 sessions over four weeks had a notable effect. Approximately two thirds of study volunteers were men, but participant sex was not always specified. However, studies comparing response by sex reported no significant difference between men and women [471,481,483,497]. One study compared different protocols of SIT and reported similar improvements with 10 or 30 second sprints [486]. However, the protocol was particularly intense and conducted in healthy active men and women, so is unlikely to be suitable in patient populations.

SIT or HIIT resulted in similar or greater improvements in cardiorespiratory fitness and/or work capacity than CMIE matched for total work [422,445,451,466-469,474-478,482,484,485,487,489,491,493,498]. One study reported a similar change despite the CMIE protocol requiring 10 times the energy output of the SIT protocol. Further, SIT produced increases in VO_{2peak} faster than CMIE [482].

Some of the improvements observed may be attributable to the fact that formerly sedentary or even moderately active individuals would be unaccustomed to near maximal efforts and thus perform below their true maximum capacity during baseline assessments. Following several sessions of repeated near maximal or supramaximal efforts these individuals may be more likely to achieve their true maximum during a followup test. Nonetheless, HIIT and SIT represent time efficient means to substantially increase cardiorespiratory fitness and work capacity over a short period of time.

3.3.2.1 Cardiovascular Effects

In patients with chronic heart failure and coronary heart disease, HIIT improved cardiac output [446,462,463] and left ventricular ejection fraction [446,462]. Increased cardiac output and left ventricular ejection fractions were also observed in sedentary middle-aged cohorts [489,491], sedentary elderly [490], and overweight young women [493]. Changes were greater with HIIT than CMIE; the latter often showing no change [446,462,489,491,493]. The only study to assess the effect of multiple bouts of HIIT on heart rate variability reported increased 24-hour heart rate variability across multiple frequencies, indicating an increase predominantly in parasympathetic activity, in elderly men after 14 weeks of HIIT [472].

3.3.2.2 Blood Pressure

Reports of blood pressure changes following multiple sessions of HIIT have been varied. In women with a family history of hypertension [502], and men and women with hypertension, HIIT reduced ambulatory blood pressure with greater reductions reported overnight [489]. However, no change in resting blood pressure was observed following HIIT in patients with a recent percutaneous coronary intervention [464], or chronic heart failure [446]. Resting diastolic and systolic blood pressure were reduced in patients with metabolic syndrome after 16 weeks of HIIT [422], but blood pressure changes after 12 weeks in a similar cohort using a similar exercise protocol did not reach statistical significance [424]. Interestingly, the second study also showed more modest increases in VO_{2peak} and flow mediated dilation indicating less overall change in this cohort [424]. Neither study clearly specified when measurements were taken relative to the last bout of

exercise. HIIT reduced resting systolic and/or diastolic blood pressure in two sedentary cohorts after 12 weeks [474,490]. However, no effect was observed in overweight men after two weeks [473], eight weeks [475], 12 weeks [476], or in elderly men after 14 weeks [472]. It is notable that the only study to report blood pressure at multiple time points following the last bout of exercise observed a reduction relative to baseline after 24 but not 72 hours [479]. Comparisons of HIIT and SIT with CMIE showed similar changes irrespective of exercise approach [422,424,446,474-476,502]. However, ambulatory monitoring suggests HIIT is superior to CMIE for blood pressure reduction [489].

3.3.2.3 Haemodynamics

Multiple haemodynamic parameters were reported across studies. Flow-mediated dilation was routinely substantially increased following HIIT in a range of populations, and did so to a similar or greater extent than CMIE [422,424,465,466,469,489,499]. Conversely, SIT did not change flow-mediated dilation in chronic heart failure patients unless combined with resistance training [438]. Fourteen weeks of HIIT increased spontaneous cardiac baroreflex in elderly men [472], and SIT improved peripheral artery distensibility in already active young men and women [499]. Thus, HIIT and SIT appear to provide time efficient means to improve haemodynamic parameters in multiple clinical populations.

3.3.2.3 Summary of Cardiovascular and Cardiorespiratory effects of High-Intensity Intermittent and Sprint Interval Training

HIIT and SIT is a time efficient and effective means of rapidly increasing fitness and aerobic work capacity. Very limited data from patients with heart disease suggest that some of these cardiac output is improved. Reports on reduction of blood pressure are inconsistent with population and measurement time following exercise being the likely explanatory factors. The more consistently reported improvements in flow-mediated dilation and other blood pressure related parameters support a therapeutic role of HIIT in hypertensive populations. Improvements following HIIT were predominantly shown to be similar or greater than those reported for CMIE.

Table 3.2: Cardiorespiratory and Cardiovascular Effects of High Intensity Intermittent and Sprint Interval Training

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
446	9 men & 5 women, chronic heart failure, 68±6 years, BMI 24±n.r., VO_{2peak} 16±4	**HIIT**: 5 x 3 min cycling intervals at 80% VO_{2peak} with 3 active recovery periods at 40% VO_{2peak} x 3/wk x 12 wk	VO_{2peak} ↑23%, WR_{max} ↑29%, Q_{max} ↑31%, LVEF ↑27% vs baseline/CME/CON; SBP & DBP n.s.
	8 men & 5 women, chronic heart failure, 66±8 years BMI 24±n.r., VO_{2peak} 16±3	**CME**: 30 min cycling at 60% VO_{2peak} x 3/wk x 12 wk	VO_{2peak}, WR_{max}, Q_{max}, LVEF, SBP & DPB, n.s.
	9 men & 4 women, chronic heart failure, 68±9 years, 25±n.r., VO_{2peak} 21±5	**CON**: usual care with advice for home-based physical activity	VO_{2peak} & WR_{max} n.s.; Q_{max} ↓17% vs baseline & HIIT; LVEF, SBP & DBP n.s.
438	12 men & 2 women, chronic heart failure, 52±11 years, BMI 29±5, VO_{2peak} 16±4	**SIT**: 30 s cycle intervals at 50% W_{max} with 60 s passive recovery for 40 min x 3/wk x 12 wk	VO_{2peak} ↑9.6%, W_{max} ↑14% vs baseline; FMD n.s.
	12 men & 2 women, chronic heart failure, 54±10 years, BMI 28±4, VO_{2peak} 16±6	**SIT&RET**: 30 s cycle intervals at 50% W_{max} with 60 s passive recovery for 20 min & 20 min of resistance exercises (3 sets of 12 repetitions for hip flexors & extensors, biceps, & shoulders) x 3/wk x 12 wk	VO_{2peak} ↑17%, W_{max} ↑18% vs. baseline; FMD ↑63% vs baseline & SIT
447	13 men & 3 women, post stroke, 54±9 years, BMI 25±n.r., VO_{2peak} 19±4	**HIIT:** 4 min cycling at 40% of the max workload & 1 min at 80%, repeated 6 times x 3/wk x 12 weeks	VO_{2peak} 15%, 6-min walk test distance ↑16%
462	6 men & 2 women, coronary heart disease, 61±4 years, BMI 26±3, VO_{2peak} 27±5	**HIIT**: 4 x 4 min incline treadmill walking at 85-95% HR_{peak} with 3 min active recovery periods at 60-70% HR_{peak} x 8 wk (sessions/week: 5/5/5/5/3/3/3/3)	VO_{2peak} ↑17%, W_{max} ↑25%, Q_{max} ↑29% & LVEF ↑5% (p=0.06) vs baseline & RET
	10 men, coronary heart disease, 67±6 years, BMI 27±3, VO_{2peak} 29±4	**RET**: 4 sets of 4 repetitions of horizontal leg press at 85-90% 1RM 3/wk x 8 wk	VO_{2peak}, W_{max}, Q_{max} & LVEF n.s.

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
463	7 men & 3 women, coronary heart disease, 63±7 years, BMI 26±3, VO_{2peak} 27±5	**HIIT**: 4 x 4 min incline treadmill walking at 85-95% HR_{peak} with 3 min active recovery periods at 60-70% HR_{peak} x 3/wk x 10 wk	VO_{2peak} ↑16%, W_{max} ↑32%, $Q_{80\% VO2peak}$ ↑23% vs baseline vs baseline
	6 men & 2 women, coronary heart disease, 61±7 years, BMI 27±3, VO_{2peak} 26±4	**HIIT+O_2**: 4 x 4 min incline treadmill walking at 85-95% HR_{peak} with 3 min active recovery periods at 60-70% HR_{peak} x 3/wk x 10 wk with 100% O_2 enriched air provided during exercise	VO_{2peak} ↑15%, W_{max} ↑29%, $Q_{(80\% VO2peak)}$ ↑28 vs baseline
445	8 men, post infarction heart failure, 62±8, BMI 28±2, VO_{2peak} 19±5	**HIIT**: 2-4 x 4 min incline treadmill walking at 75-80% HRR with 3 min active recovery at 45-50% HRR 3-5/wk x 12 week (sessions/week:2/2/2/3//3/3/4/4/4/5/5/5)	VO_{2peak} ↑22% vs baseline
	8 men, post infarction heart failure, 63±9, BMI 27±3, VO_{2peak} 18±4	**CME**: 30-45 min incline treadmill walking at 45-60% HRR 3-5/wk x 12 week (sessions/week:2/2/2/3//3/3/4/4/4/5/5/5) with variables matched to the HIIT group week by week	VO_{2peak} ↑22% vs baseline
464, 465	17 men & 3 women following percutaneous coronary intervention, 57±10 years, BMI 27±4, VO_{2peak} 23±6	**HIIT**: 4 x 4 min incline treadmill walking at 80-90% HR_{max} with 3 min active recovery periods at 60-70% HR_{max} 3/wk x 26 wk	VO_{2peak} ↑17% & FMD ↑338% vs. baseline & CON; SBP & DBP n.s.
	16 men & 4 women following percutaneous coronary, 61±10 years, BMI 28±3, VO_{2peak} 23±6	**CON**: usual care	VO_{2peak} ↑8%; SBP & DBP n.s.
466, 467	24 men & 4 women, following CABG, 60±7 years, BMI 26±6, VO_{2peak} 27±5	**HIIT:** 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 5/wk x 4 wk supervised, then advised to continue at home	At 4 wk: VO_{2peak} ↑12% & FMD ↑45% vs. baseline At 6 months: VO_{2peak} ↑18%; FMD declined to baseline
	24 men & 7 women, following CABG, 62±8 years, BMI 28±4, VO_{2peak} 26±5	**CME:** 47 min at 70% HRmax 5/wk for 4 wk (matched for energy expenditure with HIIT), then advised to continue at home	At 4 wk: VO_{2peak} ↑9% & FMD ↑34% At 6 months: VO_{2peak} ↑13%; FMD declined to baseline

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
468, 469	25 men & 5 women, post myocardial infarction, 57±10 years, BMI 27±3, VO_{2peak} 32±6	**HIIT:** 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 3/wk x 12 wk	At 12 weeks: VO_{2peak} ↑15% vs baseline & group exercise At 6 months: decline in VO_{2peak} At 30 months: further decline in VO_{2peak} but still above baseline
	49 men & 10 women, post myocardial infarction, 58±9 years, BMI 27±4, VO_{2peak} 32±7	**Group session:** 10 min warmup, 35 min aerobic exercise (walking/jogging/lunges), 15 min cool down, stretching & relaxation x 3/wk x 12 wk	At 12 weeks: VO_{2peak} ↑8% vs baseline At 6 months: n.s. decline in VO_{2peak} At 30 months: further decline in VO_{2peak} to baseline
470	8 (sex n.r.), type 2 diabetes, 63±8 years, BMI 32±6, VO_{2peak} n.r.	**HIIT**: 10 x 1 min intervals at ~90% HR_{max} with 1 min rest periods 3/wk x 2 wk	VO_{2peak} n.r., $W_{max,}$ ↑10%
471	16 men, elderly, 65±4 years, BMI 21±n.r., VO_{2peak} 27±5	**HIIT**: 6 x 1 min cycling at VT_2 with active recovery at VT_1 2/wk x 9 wk	VO_{2peak} ↑11%, WR_{max} ↑15% vs baseline
	19 women, elderly, 65±4 years, BMI 25±n.r., VO_{2peak} 19±4		VO_{2peak} ↑13%, WR_{max} ↑21% vs baseline & men
472	11 men, elderly, 74±4 years, BMI 28±4, VO_{2peak}	**HIIT**: 9 x 1 min cycling intervals at 85% HRR with 4 min active recovery at 65% HRR x 4/wk x 14 wk	VO_{2peak} ↑19%; R-$R_{daytime}$ ↑12%, R-R_{24hr} 7.7%, slSBR ↑40%; SBP & DBP n.s.
422, 423, 425	4 men & 7 women, metabolic syndrome, 55±13 years, BMI 30±2, VO_{2max} 34±3	**HIIT**: 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 3/wk x 16 wk	VO_{2peak} ↑35% vs baseline/CME/CON; SBP ↓6.3%, DBP ↓6.3% & FMD ↑180% vs. baseline & CON
	4 men & 4 women, metabolic syndrome, 52±11 years, BMI 29±2, VO_{2max} 36±3	**CME**: 47 min at 70% HR_{max} 3/wk for 16 wk (matched for energy expenditure with HIIT)	VO_{2peak} ↑16%; , SBP ↓7.6%, & FMD ↑80% vs baseline & CON
	5 men & 4 women, metabolic syndrome, 50±9 years, BMI 32±1, VO_{2max} 32±3	**CON**: no intervention	VO_{2peak}, SBP & DBP n.s.

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
424	11 (sex n.r.), metabolic syndrome, 50±10 years, BMI 31±4, VO_{2peak} 34±10	**HIIT**: 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 3/wk x 12 wk	VO_{2peak} ↑11% & FMD ↑~28% vs baseline; SBP, DBP n.s.
	11 (sex n.r.), metabolic syndrome, 51±8 years, BMI 32±4, VO_{2peak} 32±5	**RET**: 3 sets of 8-12 repetitions x 3-5 exercises x 3/wk x 12 wk	VO_{2peak} n.s.; FMD ↑~28% vs baseline; SBP, DBP n.s.
	10 (sex n.r.), metabolic syndrome, 53±10 years, BMI 30±4, VO_{2peak} 28±6	**HIIT+RET**: HIIT as above 2/wk, 3 resistance exercises for 8-12 repetitions 1/wk, all for 12 wk	VO_{2peak} ↑10%; FMD ↑~38% vs baseline; SBP, DBP n.s.
	11 (sex n.r.), metabolic syndrome, 47±10 years, BMI 32±4, VO_{2peak} 34±10	**CON**: no exercise	VO_{2peak}, FMD SBP & DBP n.s.
502	11 women, familial history of hypertension, 24±4 years, BMI 24+5, VO_{2max} 29±4, BP 106/65	**HIIT**: walking: 1 min at 80-90% VO_{2max} 2 min walking at 50-60% for 40 min + 15 min calisthenics 3/wk x 16 wk	VO_{2max} ↑16% vs baseline & CME, HRR ↑75% vs baseline & CON; 24 hr SBP ↓1.8% & DBP ↓2.8% vs baseline, daytime BP n.s., nighttime SBP ↓3.8% & DBP ↓5.8. vs baseline
	11 women, familial history of hypertension, 27±5 years, BMI 24±5, VO_{2max} 30±4, BP 105/65	**CME**: walking at 60-70% VO2max for 40 minutes + 15 min calisthenics 3/wk x 16 wk	VO_{2max} ↑8% vs baseline, HRR n.s.; 24 hr SBP ↓2.4% & DBP ↓3.0% vs baseline, daytime BP n.s., nighttime SBP ↓3.4% & DBP ↓5.0% vs baseline
	9 women, familial history of hypertension, 25±4 years, BMI 24±4, VO_{2max} 30±4, BP 106/62	**CON**: No intervention	VO_{2max}, HRR, 24 hr, daytime & nighttime BP n.s.
473	12 men, overweight/obese, 24±5 years, BMI 29±3, VO_{2peak} 38±6	**HIIT**: 6 x 4 min cycling at 85% VO_{2peak}, with 2 min rest x 6 sessions over 14 d.	VO_{2peak} ↑8.3%; SBP & DBP n.s.

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
474	8 men, sedentary, 37±8 years, BMI n.r. (BW 96±11 kg), VO_{2peak} 36±5	**HIIT**: 5 x 2 min running >95% HR_{max} with 2 min rest periods 3/wk x 12 wk	VO_{2max} ↑14% vs baseline & other groups; SBP ↓6.2% vs baseline; DBP n.s.
	9 men, sedentary, 31±6 years, BMI n.r. (86±16 kg), VO_{2peak} 39±7	**CME**: 60 min running at 80% HR_{max} (~65% VO_{2peak}) 3/wk x 12 wk	VO_{2max} ↑7%, SBP ↓6.1% & DBP ↓6.2% vs baseline
	8 men, sedentary, 36±6 years, BMI n.r. (95±24 kg), VO_{2peak} 38±9	**RET**: 60 min 3-4 sets of: squat, hack squat, incline leg pres, isolated knee extension, hamstring curls, and calf raises 3/wk x 12 wk	VO_{2max}, SBP & DBP n.s.
	11 men, sedentary, 30±6 years, BMI n.r. (87±11 kg), VO_{2peak} 39±8	**CON**: no exercise x 12 wk	VO_{2max} , SBP & DBP n.s.
475	7 (sex n.r.), sedentary overweight, 41±12 years, BMI 31±3, VO_{2peak} ~23±n.r.%	**HIIT**: 10 x 1 min cycling at 90% VO_{2peak} with 2 min recovery at 30% VO_{2peak} x 4 sessions/wk for 8 wk, & diet education (1 hour seminar)	VO_{2peak} ↑24% & time to exhaustion on exercise test ↑23% vs baseline; SBP & DBP n.s.
	6 (sex n.r.), sedentary overweight, 45±17 years, BMI 30±3, VO_{2peak} ~24±n.r.%	**CME**: 50% VO_{2peak} matched to energy expenditure of HIIT for time, & diet education (1 hour seminar).	VO_{2peak} ↑19% & time to exhaustion on exercise test ↑24% vs baseline; SBP & DBP n.s.
	8 (sex n.r.), sedentary overweight, 40±13 years, BMI VO_{2peak} ~23±n.r.%	**Diet**: diet education only (1 hour seminar).	VO_{2peak}, SBP & DBP n.s.
476	3 men & 11 women, sedentary, 47±8 years, BMI 37±4 , VO_{2peak} 24±5	**HIIT:** 4 x 4 min treadmill walking/running at 85-95% HR_{max} with 3 min active recovery periods at 50-60% HR_{max} 3/wk x 12 wk	VO_{2max} ↑33% vs baseline/CME/RET; DBP ↓7% vs. baseline/RET; SBP n.s.
	2 men & 11 women, 44±8 years, BMI 37±5, VO_{2peak} 25±5	**CME**: 47 min walking/jogging at 50–60% of HR_{max}	VO_{2max} ↑16% vs baseline; DBP ↓9% vs. baseline/RET; SBP n.s.
	3 men & 10 women, 46±10 years, VO_{2peak} 25±7	**RET**: 4 sets of 5 repetitions on leg press/squat & 3 sets of 30 repetitions on unspecified abdominal & back exercises	VO_{2max} ↑10% vs baseline; SBP & DBP n.s.

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
477	5 men & 5 women, sedentary, 18-32 years, BW 64±11, VO_{2peak} 39±9	**HIIT&SIT:** 4 sessions/wk for 15 weeks with a mix of CME at 70% HRR, SIT (15-30 s intervals) and HIIT (60-90 s intervals) sessions. Recovery periods were based on HR returning to 120-130 bpm	VO_{2peak} ↑26% vs. baseline
	8 men & 9 women, sedentary, 18-32 years, BW 61±13, VO_{2peak} 37±8	**CME**: 4 sessions/wk x 20 weeks: 30-45 min at 60-85% HRR at approximately twice the E expenditure of HIIT&SIT	VO_{2peak} ↑32% vs. baseline
478	8 (sex n.r.), sedentary overweight/obese, 40±8 years, BMI 28±4, VO_{2peak} n.r.	**HIIT:** Thrice weekly cycle ergometry at 20% above anaerobic threshold at a 2:1 exercise/rest ratio: wk 1-4 for 20, 30, 40, 50 min, respectively, then 60 min sessions x 3/wk x 12 wk	Anaerobic threshold ↑92% vs. baseline
	8 (sex n.r.), sedentary overweight/obese, 40±8 years, BMI 28±2, VO_{2peak} n.r.	**CME:** Thrice weekly cycle ergometry at 10% below anaerobic threshold: wk 1-4 for 20, 30, 40, 50 min, respectively, then 60 min sessions for 8 wk (total duration of 12 wk).	Anaerobic threshold ↑42% vs. baseline & CME
479	10 men, sedentary overweight/obese, 32±9 years, BMI 31±4, VO_{2peak} 33±1	**SIT**: 4-7 bouts of 30 s cycle sprints at 6.5% FFM resistance 3/wk for 2 wk (sprints/session: 4/4/5/5/6/6) with 4.5 min active at 30 W resistance	VO_{2peak} ↑9.5% & SBP ↓4.7%; DBP n.s. (change in SBP was only significant at 24 hr but not 72 hr post-exercise)
480	14 women, overweight/obese, 30±7 years, BMI 36±6, VO_{2max} 22±1	**SIT**: Six sessions over 28 days: 4-7 (increased by 1 every week) 30 s sprints against resistance of 5% BW with 4 min recovery between sprints.	VO_{2max} ↑13% vs baseline & CON
	14 women, overweight/obese, 31±6 years, BMI 35±6, VO_{2max} 21±1	**CON**: Maintenance of baseline physical activity	VO_{2max} n.s.

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
481	8 women, sedentary, 23±3 years, BMI 23±2, VO_{2peak} 33±2	**SIT:** cycling 10 min total containing 2 all out sprints for 10/15/20/20/20/20 s in wk 1-6 x 3/wk, respectively, remaining time was at 60 W	VO_{2peak} ↑12% vs baseline & CON
	8 women, sedentary, 22±1 years, BMI 23±2, VO_{2peak} 33±2	**CON:** no exercise	VO_{2peak} n.s.
	7 men, sedentary, 26±3 years, BMI 24±2, VO_{2peak} 36±2	**SIT:** cycling 10 min total containing 2 all out sprints for 10/15/20/20/20/20 s in wk 1-6, respectively, remaining time was at 60 W	VO_{2peak} ↑15% vs baseline & CON
	6 men, sedentary, 25±2 years, BMI 24±2, VO_{2peak} 38±3	**CON:** no exercise	VO_{2peak} n.s.
457	3 men & 6 women, sedentary, 24±4 years, BMI 25±2, VO_{2peak} 35±7	**SIT moderate:**10:20 s sprint:active recovery at 120% W_{peak} & 20 W, respectively, done for 30, 35, & 40 minutes in wk 1-2, 3-4, & 4-6, respectively x 3/wk	VO_{2peak} & FMD n.s.
	4 men & 7 women, sedentary, 23±3 years, BMI 23±3, VO_{2peak} 41±8	**SIT heavy:** 30:60 s sprint:active recovery at 120% W_{peak} & 20 W, respectively, done for 30, 35, & 40 minutes in wk 1-2, 3-4, & 4-6, respectively x 3/wk	VO_{2peak} ↑15% vs. baseline & SIT moderate; FMD n.s.
482	5 men & 5 women, sedentary, 24±1 years, BMI 24±n.r., VO_{2peak} 41±2 ml/kg/min	**SIT:** 4-6 bouts of 30s cycle sprints braked at 7.5% body weight 3/wk x 6 wk (bouts/session/week: 4/4/5/5/6/6/)	VO_{2peak} ↑7% (at week 3 & 6 vs baseline)
	5 men & 5 women, 23±1 years, BMI 24±n.r., VO_{2peak} 41±2 ml/kg/min	**CME**: Continuous cycling ~65% VO_{2peak} 40-60 min (length ↑10 min/fortnight) 5/wk for 6 weeks.	VO_{2peak} ↑2% &↑10% at week 3 & 6 vs baseline, respectively

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
483	11 men, active, 25±6 years, BMI 25±n.r., VO_{2peak} 46±4	**SIT**: 4-6 30 s cycle sprints against resistance of 7.5% BW with 5 min unloaded cycling between sprints (sprints per session: 4/4/5/5/6/6)	VO_{2max} ↑5.9% & W_{peak} ↓10%
	9 women, active, 25±3 years, BMI 22±n.r., VO_{2peak} 41±6		VO_{2max} ↑6.8% & W_{peak} ↓9.0%
	5 men, active, 23±3 years, BMI 25±n.r., VO_{2peak} 46±7	**CON**: before & after tests but no intervention	VO_{2max} n.s.
	4 women, active, 23±3 years, BMI 24±n.r., VO_{2peak} 39±2		VO_{2max} n.s.
484	5 men & 3 women, healthy active, 21±5 years, BMI 25±n.r., VO_{2peak} 42±6 ml/kg/min	**SIT**: Six sessions over 14 days: 4-7 30 s sprints against resistance of 7.5% BW with 4 min recovery between sprints (sprints per session: 4/5/6/6/7/7).	VO_{2peak} ↑7%,& VO_2 slow component amplitude ↓23% vs. baseline/CME/CON
	5 men & 3 women, healthy active, 20±4 years, BMI 24±n.r., VO_{2peak} 43±5 ml/kg/min	**CME**: 80 rpm continuous cycling at 80% GET for a duration matched to give work equivalent to SIT group.	VO_{2peak} & VO_2 slow component amplitude n.s.
	5 men & 3 women, healthy active, 20±1 years, BMI 23±n.r., VO_{2peak} 47±8 ml/kg/min	**CON**: Habitual physical activity.	VO_{2peak} & VO_2 slow component amplitude n.s.
485	15 women, healthy, 22±1 years, BMI 24±2, VO_{2peak} 29±8	**SIT**: 3 sessions/wk over 15 wk: 8 s sprints/12 s active (20-30 rpm) recovery at 0.5 kg resistance. Progressing from 5 min sessions to 20 min sessions with 0.5 kg resistance added once 20 minutes	VO_{2peak} ↑24% vs baseline & CON
	15 women, healthy, 21±1 years, BMI 22±1, VO_{2peak} 31±8	**CME**: 3 sessions/wk over 15 weeks: 10-20 min at 60% VO_{2peak} for 10-20 min progressing to 40 minutes	VO_{2peak} ↑20% vs baseline & CON
	15 women, healthy, 22±1 years, BMI 24±1, VO_{2peak} 31±6	**CON**: No change in physical activity over 15 wk	VO_{2peak} n.s.

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
486	35 men & 13 women, active, 24±3 years, BMI 25±n.r., VO_{2peak} n.r. (NB: data were reported for the entire cohort only with groups matched for VO_{2peak} & sex)	**SIT:** 4-6 bouts of 30 s cycle sprints 10% BW resistance with 4 min active recovery (no resistance) 3/wk for 2 wk (bouts/session: 4/4/5/5/6/6)	VO_{2peak} ↑9.3%; 5 km time trial time ↓5.2%; peak power ↑9.5% vs baseline & CON
		SIT: 4-6 bouts of 10 s cycle sprints 10% BW resistance with 4 min active recovery (no resistance) 3/wk for 2 wk (bouts/session: 4/4/5/5/6/6)	VO_{2peak} ↑9.2%; 5 km time trial time ↓3.5%; peak power ↑8.5% baseline & CON
		SIT: 4-6 bouts of 10 s cycle sprints 10% BW resistance with 2 min active recovery (no resistance) 3/wk for 2 wk (bouts/session: 4/4/5/5/6/6)	VO_{2peak} ↑3.8% (p=0.06); 5 km time trial time ↓3.0%; peak power ↑4.2% baseline & CON
		CON: no exercise	VO_{2peak}; 5 km time trial time; & peak power n.s. vs baseline
487	5 men & 5 women, healthy, 24±1 years, BMI 24±n.r., VO2peak 41±2	**SIT**: 4-6 bouts of 30 s cycle sprints 7.5% BW resistance with 4.5 min active recovery (30 W resistance) 3/wk x 6 wk (intervals/session/week: 4/4/5/5/6/6)	VO_{2peak} ↑7%
	5 men & 5 women, healthy, 23±1 years, BMI 24±n.r., VO_{2peak} 41±2	**CME:** 40-60 min cycling at 65% VO_{2peak} 5/wk x 6 wk (time/session/week: 40/50/60)	VO_{2peak} ↑10%
488	16 men, active, 22±2 years, BMI 24±3, VO_{2peak} 48±9	**SIT**: 4-6 30 s cycle sprints against resistance of 7.5% BW with 4 min unloaded cycling between sprints x 3/wk x 2 wk (sprints per session: 4/4/5/5/6/6)	Work output during time trial ↑6%

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
489	15 men & 10 women, hypertensive, 53±7 years, BMI 27±4, VO_{2peak} 36±9	**HIIT:** 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 60-70% HR_{max} 3/week x 12 weeks	VO_{2max} ↑14% vs baseline & CME; 24-hour SBP ↓7.8% & nighttime SBP ↓7.5% vs. baseline & CME; 24 hour DBP ↓8.6%, daytime SBP ↓8.1% & DBP ↓8.7%, nighttime DBP ↓6.1% vs baseline; FMD ↑64% & LVEF ↑11% vs. baseline & CME; SV ↑11% & CO ↑13% vs. baseline
	13 men & 10 women, hypertensive, 54±7 years, BMI 28±4, VO_{2peak} 34±7	**CME:** walking/running on treadmill at 70% of HR_{max}, for 47 min to ensure isocaloric training sessions to the HIIT group	VO_{2max} ↑5%, 24-hour SBP 3.2% & DBP ↓3.8%, daytime SBP ↓3.2% & DBP ↓4.3% vs. baseline; nighttime BP, FMD, SV, CO, & LVEF n.s.
	14 men & 11 women, hypertensive, 51±9 years, BMI 29±4, VO_{2peak} 35±8	**CON:** standard advice for essential hypertension, including 'regular light-moderate intensity exercise'	VO_{2max} & BP n.s. except nighttime SBP ↓3.9%; FMD, SV, CO, & EF all n.s.
490	10 men & 6 women, sedentary seniors, 72±2 years, BMI 25±1, VO_{2max} 33±6	**HIIT:** 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 60-70% HR_{max} 3/wk x 12 wk	VO_{2max} ↑14%, SBP ↓12%, DBP ↓8.8%, SV ↑9% & LVEF ↑13%; CO n.s.
491	7 men, sedentary, 47±7 years, BMI 26±†; 4 women, sedentary, 42±6 years, BMI 21±1, VO_{2peak} ~29 (combined men & women)	**HIIT:** 1 min intervals at 90% W_{max} with 3 min active recovery at 1st ventilatory threshold x 3/wk x 8 wk; total duration 20, 25, 30, & 35 min in wk 1-2, 2-4, 5-6, & 7-8, respectively	VO_{2peak} ↑15%; Q_{max} ↑11%, SV ↑8%, & arteriovenous oxygen difference ↑16% vs. baseline
		CME: intensity = (4 x 1st ventilatory threshold + 90% W_{max})/5 to match energy expenditure & exercise time with HIIT	VO_{2peak} ↑9% & arteriovenous oxygen difference ↑10% vs baseline; Q_{max} & SV n.s.
492	20 men, sedentary & overweight, 25±5 years, BMI 28±1, VO_{2peak} 34±1	**SIT:** 8 s sprints with 12 s recovery periods at a cadence of 120-130 & 40, respectively, for 20 min at 80-90% HR_{peak} x 3/wk for 12 w	VO_{2peak} ↑15% vs baseline & CON
	18 men, sedentary & overweigh, 25±4 years, BMI 29±1, VO_{2peak} 29±1	**CON:** no exercise	VO_{2peak} n.s.

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
493	17 women, BF >30%, 20±1 years, BMI 28±2, VO_{2peak} 33±4	**HIIT:** 5 x 3 min of track running at 85% VO_{2max} with 3 min active recovery at at 50% VO_{2max} 5/wk x 12 wk	VO_{2max} ↑8.4% vs baseline/CON/CME; LVEF ↑4.7% vs. baseline & CON
	16 women, BF >30%, 19±1 years, BMI 28±2, VO_{2peak} 33±5	**CME:** 40 min walking/jogging at 50% VO_{2max} 5/wk x 12 wk	VO_{2max} ↑4.8% & LVEF ↑1.5% vs CON
	19 women, BF >30%, 20±1 years, BMI 29±2, VO_{2peak} 33±4	**CON:** no exercise	VO_{2max} & LVEF n.s.
494	2 men & 8 women, obese, 37±10 years, BMI 32±4, VO_{2peak} 27±5	**HIIT+Diet:** Up to 10 x 4 minutes cycling at 90% $VO2_{peak}$ with 2-3 minutes rest periods, for 6 supervised sessions over 14 days. (see below for diet)	VO_{2peak} ↑16%
	3 men & 6 women, obese, 41±14 years, BMI 32±3, VO_{2peak} 26±9	**Diet**: 75% of food record estimated requirement (35% CHO)	VO_{2peak} n.s.
495	7 men, active, 20-40 years, BMI 25±3, VO_{2peak} 37±7	**HIIT**: 4 x 4 min treadmill running at 90% VO_{2peak} with min recovery periods at 60% VO_{2peak} 3/wk x 8 wk	VO_{2peak} ↑20% vs baseline & CON
	8 men, active, 20-40 years, BMI 23±2, VO_{2peak} 40±16	**CON**: no intervention	VO_{2peak} n.s.
496	20 men, active, 30±5 years, BMI 23±4, VO_{2peak} n.r.	**HIIT**: 4 x 800 m runs at ~90% age predicted HR_{max} (220-age) with a 1:1 work-to-rest ratio 3/wk x 8 wk	time to run 2.4 km ↓9.2% vs baseline & CON
	16 men, active, 30±5 years, BMI 23±2, VO_{2peak} n.r.	**CON**: no vigorous exercise	time to run 2.4 km n.s. vs baseline

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
461	9 women, active, 23±3 years, BMI 22±1, VO_{2peak} 41±5	**HIIT1:** 1 min cycling bouts at MTP x 9 with 4 min active recovery periods at VT x 3/wk x 6 wk	VO_{2peak} ↑6.5%; WR_{max} ↑20% vs baseline & CON
	9 women, active, 22±3 years, BMI 21±2, VO_{2peak} 43±5	**HIIT2**: 1 min cycling bouts x 9 with 4 min active recovery periods at VT x 3/wk x 6 wk with intensity based on a 10 point RPE scale	VO_{2peak} ↑9.9%; WR_{max} ↑12% vs baseline & CON
	9 women, active, 23±2 years, BMI 22±2, VO_{2peak} 42±5	**CON**: no exercise	VO_{2peak} & WR_{max} n.s.
497	7 women, active, 23±7 years, BMI 23±n.r., VO_{2peak} 40±n.r.	**HIIT**: 3 x 2 min cycling intervals at ~100% VO_{2peak} with 6 min recovery x 3/wk x 8 wk	After 4 wk: time to exhaustion ↑29% vs baseline After 8 wk: VO_{2peak} n.s., W_{max} ↑11%, time to exhaustion ↑56% vs baseline
	7 men, active, 24±4 years, BMI 23±n.r., VO_{2peak} 44±n.r.		After 4 wk: time to exhaustion ↑31% vs baseline After 8 wk: VO_{2peak} ↑7.9% vs baseline & women, W_{max} ↑11%, time to exhaustion ↑73% vs baseline
498	12 men, active, 25±4 years, BMI n.r. (BW 83±7 kg), VO_{2peak} 44±n.r. (NB: volunteer demographics were not provided by intervention group)	**HIIT:** 8-12 1 min cycling bouts at 120% WR_{max} x 8 sessions (intervals/session: 8/8/9/9/10/10/12/12)	VO_{2peak} ↑4.3%; WR_{max} ↑8.8% vs. baseline
		CME: 90-120 min cycling at 65% VO2peak x 8 sessions (time/session: n.r.)	VO_{2peak} ↑7.0%; WR_{max} ↑8.4% vs. baseline
499	5 men & 5 women, healthy, 23±3 years, BMI 24±2, VO_{2peak} 41±2	**SIT:** 4-6 bouts of 30s cycle sprints braked at 7.5% body weight 3/wk for 6 wk (bouts/session/week: 4/4/5/5/6/6/)	VO_{2peak} ↑7%, FMD ~↑17% & popliteal artery distensibility ↑~60% vs. baseline; carotid artery distensibility n.s.
	5 men & 5 women, healthy, 24±3 years, BMI 24±2, VO_{2peak} 41±2	**CME:** Continuous cycling ~65% VO_{2peak} 40-60 min (length ↑10 min/fortnight) 5/wk for 6 weeks.	VO_{2peak} ↑10%, FMD ~↑40%; popliteal artery distensibility ↑~57% vs baseline; carotid artery distensibility n.s.
500	5 men & 3 women, active, 24±1 years, BMI 23±n.r., VO_{2peak} 45±n.r.	**HIIT:** 4 min cycle intervals at ~90% VO_{2peak} x 10 intervals with 2 min rest 3/wk x 6 wk	VO_{2peak} ↑9%

Reference	Baseline Population	Protocol	Cardiorespiratory and Cardiovascular Changes
470	7 men, active, 21±1 years, BMI n.r. (BW 83±4 kg), VO_{2peak} n.r.	**HIIT**: 8-12 x 1 min cycling at VO_{2peak} with 75 s active recovery against 30 W resistance x 2 weeks (intervals per session: 8/8/10/10/12/12)	750 kJ exercise test: time to complete ↓9%, mean power ↑10%
501	10 women, active, 22±1 years, BMI n.r. (BW 65±2 kg), VO_{2peak} ~43±n.r.	**HIIT**: 10 x 4 min cycling at 90% VO_{2peak} with 2 min active rest periods 3/wk x 6 wk	Average power output by wk (1 vs. 2, 1 vs. 3… 1vs. 6): ↑9.1%, ↑19%, ↑23%, ↑28%, ↑33%.

Study population demographics (sample size by sex, health or training status, body mass index in kg/m2, and either VO_{2peak} or VO_{2max} in mL/kg/min, as reported by study authors, are provided as means ± standard deviation, where reported, rounded to nearest whole number. Sample sizes are based on those included in the final analysis. Results have been converted to percentage change from baseline if the change was statistically significant followed by indication if this was significant relative to comparison group(s). Results are provided to two significant figures. Where data was reported in graph form it may not have feasible to accurately calculate percentage change so ~ is used to indicate this. The protocol column contains the core exercise but does not describe warm up and cool down protocols, which consisted predominantly of 5-10 minutes periods of light-to-moderated intensity activity. **Abbreviations**: ABP, ambulatory blood pressure; AUGC, area under the glucose curve; AUIC, area under the insulin curve; AUNC, area under the non-esterified fatty acid curve; BF, body fat; BMI, body mass index (kg/m2); BP, blood pressure systolic/diastolic mm Hg; BW, body weight; CABG, coronary artery bypass graft; CGM, continuous glucose monitoring; CO, cardiac output; CON, control group; DBP, diastolic blood pressure; FMD, flow mediated dilations; GET, gas exchange threshold; hr, hour; HRR, heart rate reserve; hsCRP , high sensitivity c-reactive protein; HDL, high-density lipoprotein; HIIT, high intensity intermittent training group; IS, insulin sensitivity; ISI; insulin sensitivity index; LVEF, left ventricular ejection fraction; min, minutes; MTP, maximum tolerated power (the maximum power output that could be sustained for 1 minute); NEFA, non-esterified fatty acids; n.r., not reported; n.s., no statistically significant change/difference; OGTT, oral glucose tolerance test; OGIS, insulin sensitivity calculated from 2 hour oral glucose tolerance test; s, seconds; Q, cardiac output; RER, respiratory exchange ratio at rest; RET, resistance exercise training group; RM, repetition maximum; RPE, rate of perceived exertion; RQ, respiratory quotient; SBP, systolic blood pressure; SIT, sprint interval training group; slSBR; spontaneous cardiac baroreflex; SV, stroke volume; TAG, triacylglycerol; TC, total cholesterol; TPR, total peripheral resistance; VLDL, very low-density lipoprotein; VT, ventilatory threshold; W_{max}, maximum workload; WR, work rate.

3.3.3 Weight and Body Composition Changes with High-Intensity Intermittent and Sprint Interval Training

The 27 studies reporting body weight and/or composition changes following HIIT and SIT are summarised in *Table 3.3.* Of these 27 (n=423), 11 (n=151) reported weight or BMI reductions of 1.1-8.4% [422,425,464,465,476,478,479,485,490,492-494,498,503], and the rest (n=272) no change [424,446,466-471,473-475,477,481,486,496,500,502,504]. However, the 8.4% reduction was an extreme outlier as all other studies reported reductions of 0-2.5%. Further, one study included caloric restriction but only reported a 1.1% weight reduction over two weeks [494]. Fourteen studies reported some measure of fat mass, and of these eight reported a reduction [424,476-478,485,492-494], and six no change [471,473,474,486,496,504]. Collectively, 13 studies (n=172) showed a weight reduction and/or fat reduction. The only two studies to compare genders both reported no change in either body weight or fat mass [471,481]. One study, with a female only cohort, showed that fat reduction was greater in those with a higher fat mass at baseline [485].

Thirteen studies compared HIIT/SIT with CMIE for weight change [422,423,425,446,451,466-469,474-478,485,493,494,498]. Seven reported no weight change in either group [424,446,451,466-469,475,477], five reported largely comparable modest changes of 1-3.9% [422,425,474,478,485,498,503], and one reported greater weight reductions of 8.4% and 5.9% in the HIIT and CMIE group, respectively [493]. This larger reduction followed 12 weeks of track running five times per week. This is in contrast to the majority of studies, which assessed thrice weekly training on cycle ergometers or treadmills.

Seven studies compared HIIT/SIT with CMIE for change in body composition [474-478,485,493]. Six reported reductions in the HIIT/SIT and/or CMIE groups relative to baseline with either no significant difference between groups or slightly greater reductions with HIIT/SIT [474,476-478,485,493].

Given the heterogeneity of study designs and cohorts, it was not feasible to do statistical analysis to ascertain the reason for the discrepancy. However, data on weight, body composition, fitness of volunteers, sample size, and duration of

each intervention were tabulated in a spreadsheet to facilitate informal analysis. Studies reporting weight and/or fat mass reduction tended to be longer or contain volunteers with a higher mean VO_{2peak}. However, this conclusion requires confirmation via more rigorous analysis preferably of pooled original data. It is noteworthy that any weight and body composition changes following HIIT/SIT were not only comparable in magnitude to CMIE, but were achieved with <50% of the time investment.

Table 3.3: Weight and Body Composition after High-Intensity Intermittent and Sprint Interval Training

Reference	Baseline Population	Protocol	Weight & Body Composition
421	8 adults (sex n.r.), type 2 diabetes, 63±8 years, BMI 32±6, VO_{2peak} n.r.	**HIIT**: 10 x 1 min intervals at ~90% HR_{max} with 1 min rest periods 3/wk x 2 wk	BW n.s.
422, 423, 425	4 men & 7 women, metabolic syndrome, 55±13 years, BMI 30±2, VO_{2max} 34±3	**HIIT**: 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 3/wk x 16 wk	BW ↓2.5%
	4 men & 4 women, metabolic syndrome, 52±11 years, BMI 29±2, VO_{2max} 36±3	**CME**: 47 min at 70% HR_{max} 3/wk for 16 wk (matched for energy expenditure with HIIT)	BW ↓3.9%
	5 men & 4 women, metabolic syndrome, 50±9 years, BMI 32±1, VO_{2max} 32±3	**CON**: no intervention	BW n.s.
424	11, metabolic syndrome, 50±10 years, BMI 31±4, VO_{2peak} 34±10	**HIIT**: treadmill walking/running at 90-95% HR_{peak} for 4 min x 4 bouts with active recovery periods of 3 min at 70% HR_{peak} x 3/wk x 12 wk	BW n.s.; FM ↓7% vs. baseline; FFM n.s.
	11, metabolic syndrome, 51±8 years, BMI 32±4, VO_{2peak} 32±5	**RET**: 3 sets of 8-12 repetitions x 3-5 exercises x 3/wk x 12 wk	BW n.s.; FM ↓6% vs. baseline; FFM n.s.
	10, metabolic syndrome, 53±10 years, BMI 30±4, VO_{2peak} 28±6	**HIIT+RET**: HIIT as above 2/wk, 3 resistance exercises for 8-12 repetitions 1/wk, all for 12 wk	BW & FM n.s.; FFM ↑ n.r. vs. baseline
	11, metabolic syndrome, 47±10 years, BMI 32±4, VO_{2peak} 34±10	**CON**: no exercise	BW & FM n.s.; FFM ↑ n.r. vs. baseline
446	9 men & 5 women, chronic heart failure, 68±6 years, BMI 24±n.r., VO_{2peak} 16±4	**HIIT**: 5 x 3 min cycling intervals at 80% VO_{2peak} with 3 active recovery periods at 40% VO_{2peak} x 3/wk x 12 wk	BW n.s
	8 men & 5 women, chronic heart failure, 66±8 years BMI 24±n.r., VO_{2peak} 16±3	**CME**: 30 min cycling at 60% VO_{2peak} x 3/wk x 12 wk	BW n.s.
	9 men & 4 women, chronic heart failure, 68±9 years, 25±n.r., VO_{2peak} 21±5	**CON**: usual care with advice for home-based physical activity	BW n.s

Reference	Baseline Population	Protocol	Weight & Body Composition
464, 465	25 men & 5 women, post myocardial infraction, 57±10 years, BMI 27±3, VO_{2peak} 32±6	**HIIT:** 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 3/wk x 12 wk	BW n.s At 6 months: BW n.s. At 30 months: BW n.s.
	49 men & 10 women, post myocardial infraction, 58±9 years, BMI 27±4, VO_{2peak} 32±7	**Group session:** 10 min warmup, 35 min aerobic exercise (walking/jogging/lunges), 15 min cooldown, stretching & relaxation 3/wk x 12 wk	BW n.s. At 6 months: BW n.s. At 30 months: BW n.s.
466, 467	24 men & 4 women, following CABG, 60±7 years, BMI 26±6, VO_{2peak} 27±5	**HIIT**: 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 5/wk x 4 wk supervised, then advised to continue at home	At 4 wk: BW n.s. At 6 months: BW n.s.
	24 men & 7 women, following CABG, 62±8 years, BMI 28±4, VO_{2peak} 26±5	**CME**: 47 min at 70% HRmax 5/wk for 4 wk (matched for energy expenditure with HIIT), then advised to continue at home	At 4 wk: BW n.s. At 6 months: BW n.s.
479	10 men, sedentary overweight/obese normoglycaemic/tensive, 32±9 years, BMI 31±4, VO_{2peak} 33±1	**SIT**: 4-7 bouts of 30 s cycle sprints at 6.5% FFM resistance 3/wk for 2 wk (sprints/session: 4/4/5/5/6/6) with 4.5 min active at 30 W resistance	BW ↓1.1% (p=0.055)
475	7 (sex distribution matched but n.r.), sedentary overweight, 41±12 years, BMI 31±3, VO_{2peak} ~23±n.r.	**HIIT**: 10 x 1 min cycling at 90% VO_{2peak} with 2 min recovery at 30% VO_{2peak} x 4 sessions/wk for 8 wk, & diet education (1 hour seminar)	BW & FM n.s.
	6 (sex distribution matched but n.r., sedentary overweight, 45±17 years, BMI 30±3, VO_{2peak} ~24±n.r.	**CME**: 50% VO_{2peak} matched to energy expenditure of HIIT for time x 4 sessions/wk for 8 wk, & diet education (1 hour seminar).	BW & FM n.s.
	8 (sex distribution matched but n.r.), sedentary overweight, 40±13 years, BMI VO_{2peak} ~23±n.r.	**Diet**: diet education only (1 hour seminar).	BW & FM n.s.
494	2 men & 8 women, 37±10 years, BMI 32±4, VO_{2peak} 27±5	**HIIT+Diet:** Up to 10 x 4 minutes cycling at 90% $VO2_{peak}$ with 2-3 minutes rest periods, for 6 supervised sessions over 14 days. (see below for diet)	BW ↓1.1% & FM ↓2.6% vs baseline; FFM ↑2.1% vs baseline & Diet
	3 men & 6 women, 41±14 years, BMI 32±3, VO_{2peak} 26±9	**Diet**: 75% of food record estimated requirement (35% CHO)	BW ↓2.2%, FM ↓2.4% & FFM ↓2.1% vs baseline

Reference	Baseline Population	Protocol	Weight & Body Composition
473	12 men, overweight/obese, 24±5 years, BMI 29±3, VO_{2peak} 38±6	**HIIT**: 6 x 4 min cycling at 85% VO_{2peak}, with 2 min rest x 6 sessions over 14 d.	BW n.s.; WC ↓1.4%
496	20 men, active, 30±5 years, BMI 23±4, VO_{2peak} n.r.	**HIIT**: 4 x 800 m runs at ~90% age predicted HR_{max} (220-age) with a 1:1 work-to-rest ratio 3/wk x 8 wk	BW & BF% n.s.
	16 men, active, 30±5 years, BMI 23±2, VO_{2peak} n.r.	**CON**: no vigorous exercise	BW & BF% n.s.
492	20 men, sedentary & overweight, 25±5 years, BMI 28±1, VO_{2peak} 34±1	**SIT**: 8 s sprints with 12 s recovery periods at a cadence of 120-130 & 40, respectively, for 20 min at 80-90% HR_{peak} x 3/wk for 12 w	BW ↓1.7%, FM ↓6.7%, visceral fat area ↓10% vs baseline & CON
	18 men, sedentary & overweight, 25±4 years, BMI 29±1, VO_{2peak} 29±1	**CON**: no exercise	BMI, FM & visceral fat area n.s.,
474	8 men, sedentary, 37±8 years, BMI n.r. (BW 96±11 kg), VO_{2peak} 36±5	**HIIT**: 5 x 2 min running >95% HR_{max} with 2 min rest periods 3/wk x 12 wk	BW & BF% n.s.
	9 men, sedentary, 31±6 years, BMI n.r. (86±16 kg), VO_{2peak} 39±7	**CME**: 60 min running at 80% HR_{max} (~65% VO_{2peak}) 3/wk x 12 wk	BW ↓1.2%, BF% ↓7.0% vs baseline
	8 men, sedentary, 36±6 years, BMI n.r. (95±24 kg), VO_{2peak} 38±9	**RET**: 60 min 3-4 sets of: squat, hack squat, incline leg pres, isolated knee extension, hamstring curls, and calf raises 3/wk x 12 wk	BW ↑1.8% vs. baseline; BF% n.s.
	11 men, sedentary, 30±6 years, BMI n.r. (87±11 kg), VO_{2peak} 39±8	**CON**: no exercise x 12 wk	BW & BF% n.s.

Reference	Baseline Population	Protocol	Weight & Body Composition
481	8 women, sedentary, 23±3 years, BMI 23±2, VO_{2peak} 33±2	**SIT:** cycling 10 min total containing 2 all out sprints for 10/15/20/20/20/20 s in wk 1-6, respectively, remaining time was at 60 W	BW n.s.
	8 women, sedentary, 22±1 years, BMI 23±2, VO_{2peak} 33±2	**CON:** no exercise	BW n.s.
	7 men, sedentary, 26±3 years, BMI 24±2, VO_{2peak} 36±2	**SIT:** cycling 10 min total containing 2 all out sprints for 10/15/20/20/20/20 s in wk 1-6, respectively, remaining time was at 60 W	BW n.s.
	6 men, sedentary, 25±2 years, BMI 24±2, VO_{2peak} 38±3	**CON:** no exercise	BW n.s.
485	15 healthy women, 22±1 years, BMI 24±2, VO_{2peak} 29±8	**SIT**: 3 sessions/wk over 15 weeks: 8 s sprints/12 s active (20-30 rpm) recovery at 0.5 kg resistance. Progressing from 5 min sessions to 20 min sessions with 0.5 kg resistance added once 20 minutes was achieved	BW ↓2.4% vs. baseline & ↓4.4% vs CON, FM ↓11% vs baseline, ↓13% vs CME & ↓12.5% vs CON; FFM n.s.
	15 healthy women, 21±1 years, BMI 22±1, VO_{2peak} 31±8	**CME**: 3 sessions/wk over 15 weeks: 10-20 min at 60% VO_{2peak} for 10-20 min progressing to 40 minutes	BW & FM n.s.
	15 healthy women, 22±1 years, BMI 24±1, VO_{2peak} 31±6	**CON**: No change in physical activity	BW n.s. & FM n.s.
476	3 men & 11 women, sedentary, 47±8 years, BMI 37±4 , VO_{2peak} 24±5	**HIIT:** 4 x 4 min treadmill walking/running at 85-95% HR_{max} with 3 min recovery periods at 50-60% HR_{max} 3/wk x 12 wk	BW ↓2.0% & FM ↓2.2% vs. baseline
	2 men & 11 women, 44±8 years, BMI 37±5, VO_{2peak} 25±5	**CME**: 47 min walking/jogging at 50–60% of HR_{max}	BW ↓3.0% & FM ↓2.5% vs. baseline
	3 men & 10 women, 46±10 years, BMI 35±1, VO_{2peak} 25±7	**RET**: 4 sets of 5 repetitions on leg press/squat & 3 sets of 30 repetitions on unspecified abdominal & back exercises	BW & FM n.s.

Reference	Baseline Population	Protocol	Weight & Body Composition
451, 502	11 women, familial history of hypertension, 24±4 years, BMI 24+5, VO_{2max} 29±4, BP 106/65	**HIIT**: walking: 1 min at 80-90% VO_{2max} 2 min walking at 50-60% for 40 min + 15 min calisthenics 3/wk x 16 wk	BW n.s.
	11 women, familial history of hypertension, 27±5 years, BMI 24±5, VO_{2max} 30±4, BP 105/65	**CME**: walking at 60-70% VO2max for 40 minutes + 15 min calisthenics 3/wk x 16 wk	BW n.s.
	9 women, familial history of hypertension, 25±4 years, BMI 24±4, VO_{2max} 30±4, BP 106/62	**CON**: No intervention	BW n.s.
477	5 men & 5 women, sedentary, 18-32 years, BMI n.r. (BW 64±11 kg), VO_{2peak} 39±9	**HIIT&SIT:** 4 sessions/wk for 15 weeks with a mix of CME at 70% HRR, SIT (15-30 s intervals at 60-80% WR sustainable for 10 s) and HIIT (60-90 s intervals at 70-90% WR sustainable for 90 s) sessions. Recovery periods were based on HR returning to 120-130 bpm	BW n.s.; sum of six skinfolds ↓14.8% vs baseline & ↓9.1% vs CME
	8 men & 9 women, sedentary, 18-32 years, BW 61±13, VO_{2peak} 37±8	**CME**: 4 sessions/wk x 20 weeks: 30-45 min at 60-85% HRR at approximately twice the E expenditure of HIIT&SIT	BW n.s.; sum of six skinfolds n.s.
464, 465	17 men & 3 women following percutaneous coronary intervention, 57±10 years, BMI 27±4, VO_{2peak} 23±6	**HIIT**: 4 x 4 min incline treadmill walking at 80-90% HR_{max} with 3 min active recovery periods at 60-70% HR_{max} 3/wk x 26 wk	BMI ↓2.2% vs. baseline & CON
	16 men & 4 women following percutaneous coronary, 61±10 years, BMI 28±3, VO_{2peak} 23±6	**CON**: usual care	BMI ↑1.8%
478	8 (sex n.r.), overweight/obese, 40±8 years, BMI 28±4, VO_{2peak} n.r.	**HIIT:** cycling at 20% above AT at a 2:1 exercise/rest ratio 3/wk x 12 wk: wk 1-4 for 20, 30, 40, 50 min, respectively, then 60 min sessions for 8 wk	BMI ↓1.4 & BF%↓2% vs. baseline
	8 (sex n.r.), overweight/obese, 40±8 years, BMI 28±2, VO_{2peak} n.r.	**CME:** cycling at 10% below anaerobic threshold 3/wk x 12 wk: wk 1-4 for 20, 30, 40, 50 min, respectively, then 60 min sessions for 8 wk	BMI ↓1.5 & BF% ↓3% vs. baseline
490	10 men & 6 women, sedentary seniors, 72±2 years, BMI 25±1, VO_{2max} 33±6	**HIIT:** 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 60-70% HR_{max} 3/wk x 12 wk	BMI ↓1.6% vs. baseline

Reference	Baseline Population	Protocol	Weight & Body Composition
500	5 men & 3 women, active, 24±1 years, BMI 23±n.r., VO_{2peak} 45±n.r.	**HIIT:** 4 min cycle intervals at ~90% VO_{2peak} x 10 intervals with 2 min rest 3/wk x 6 wk	BW n.s.
486	35 men & 13 women, active, 24±3 years, BMI 25±n.r., VO_{2peak} n.r. (NB: data were reported for the entire cohort only with groups matched for VO_{2peak} & sex)	**SIT:** 4-6 bouts of 30 s cycle sprints 10% BW resistance with 4 min active recovery (no resistance) 3/wk for 2 wk (bouts/session: 4/4/5/5/6/6)	BW & BF% n.s.
		SIT: 4-6 bouts of 10 s cycle sprints 10% BW resistance with 4 min active recovery (no resistance) 3/wk for 2 wk (bouts/session: 4/4/5/5/6/6)	BW & BF% n.s.
		SIT: 4-6 bouts of 10 s cycle sprints 10% BW resistance with 2 min active recovery (no resistance) 3/wk for 2 wk (bouts/session: 4/4/5/5/6/6)	BW & BF% n.s.
		CON: no exercise	BW & BF% n.s.
489	12 men, active, 25±4 years, BMI n.r., BW 83±7 kg, VO_{2peak} 44±n.r. (NB: data were reported for the entire cohort only)	**HIIT:** 8-12 1 min cycling bouts at 120% WR_{max} x 8 sessions (bouts/session: 8/8/9/9/10/10/12/12)	BW ↓2.5% vs baseline
		CME: 90-120 min cycling at 65% VO2peak x 8 sessions (time/ session: n.r.)	BW ↓3.3% vs baseline
502	7 men, active, 20-40 years, BMI 25±3, VO_{2peak} 37±7	**HIIT**: 4 x 4 min treadmill running at 90% VO_{2peak} with min recovery periods at 60% VO_{2peak} 3/wk x 8 wk	BW & BF% n.s.
	8 men, active, 20-40 years, BMI 23±2, VO_{2peak} 40±16	**CON**: no intervention	BW & BF% n.s.
471	16 men, elderly, 65±4 years, BMI 21±n.r., VO_{2peak} 27±5	**HIIT**: 6 x 1 min cycling at VT_2 with active recovery at VT_1 2/wk x 9 wk	BW & BF% n.s.
	19 women, elderly, 65±4 years, BMI 25±n.r., VO_{2peak} 19±4		BW & BF% n.s.

Reference	Baseline Population	Protocol	Weight & Body Composition
493	17 women, BF >30%, 20±1 years, BMI 28±2, VO_{2peak} 33±4	**HIIT:** 5 x 3 min of track running at 85% VO_{2max} with 3 min active recovery at 50% VO_{2max} 5/wk x 12 wk	BW ↓8.4% & FM ↓10% vs CON & CME
	16 women, BF >30%, 19±1 years, BMI 28±2, VO_{2peak} 33±5	**CME:** 40 min walking/jogging at 50% VO_{2max} 5/wk x 12 wk	BW ↓5.9% & FM ↓5.2% vs CON
	19 women, BF >30%, 20±1 years, BMI 29±2, VO_{2peak} 33±4	**CON:** no exercise	BW & FM n.s.

Study population demographics (sample size by sex, health or activity description, body mass index in kg/m2, and either VO_{2peak} or VO_{2max} in mL/kg/min as reported by study authors in are provided as means ± standard deviation, where reported, rounded to nearest whole number. Sample sizes are based on those included in the final analysis. Results have been converted to percentage change from baseline if the change was statistically significant followed by indication if this was significant relative to comparison group(s). Results are rounded to two significant figures. Where data was reported in graph form it may not have feasible to accurately calculate percentage change so ~ is used to indicate this. The protocol column contains the core exercise but does not describe warm up and cool down protocols, which consisted predominantly of 5-10 minutes periods of light-to-moderated intensity activity. **Abbreviations**: BF%, percent body fat; BMI, body mass index (kg/m2); bpm, beats per minute; BW, body weight; CABG, coronary artery bypass graft; CON, control group; FFM, fat free mass; FM, fat mass; hr, hour or hours; HIIT, high intensity intermittent training group; min, minutes; n.r., not reported; n.s., no statistically significant change/difference; RET, resistance exercise training group; RM, repetition maximum; RPE, rate of perceived exertion; SIT, sprint interval training group; VT, ventilatory threshold; WR, work rate.

3.3.4 Metabolic Effects of High Intensity Intermittent and Sprint Interval Training

3.3.4.1 Acute Effect on Fasting and Postprandial Metabolism

Studies examining the effects of a single session of HIIT or SIT on postprandial and 24-hour metabolism are summarised in *Table 3.4*. In patients with type 2 diabetes HIIT improved 24-hour and postprandial glucose control [420]. In patients with metabolic syndrome, fasting plasma glucose was reduced for 72 hours following HIIT, whereas a reduction of comparable magnitude following CMIE was observed at 24 but not 72 hours [423]. Unsurprisingly, fasting glucose was unaffected by HIIT or SIT in individuals with normal glucose control as was insulin sensitivity 72 hours post-exercise [82,505,506]. The postprandial insulin and glucose concentrations following a high fat mixed meal were similarly unaffected by SIT in healthy active individuals [82,507]. However, both an HIIT and CMIE protocol resulted in a reduced postprandial insulin response following a high fat meal [83]. This latter cohort was larger, had a higher average VO_{2peak}, and exercised to expend 500 kcal/session on a longer protocol than the two cohorts reporting no effect of SIT. Some or all of these factors may account for the difference in findings between studies. But the most plausible explanations is that glycogen depletion is a mediator of exercise related insulin sensitisation [508-510], and depletion may have been inadequate following SIT. Both SIT and HIIT protocols reduced postprandial plasma triacylglycerol response [82,83,507]. This response was similar for CMIE when postprandial response was tested shortly after exercise [83], but was not maintained when tested the day following exercise [507], suggesting superior longevity of the effect induced by higher intensity exercise.

Table 3.4: Acute Effects of High-Intensity Intermittent and Sprint Interval Training on Metabolism

Reference	Baseline Population	Protocol	Metabolism
420	7 adults (sex n.r.), type 2 diabetes, 63±3 years, BMI 31±2, VO_{2peak} n.r.	**HIIT**: 10 x 60 s cycling at ~89% WR_{max} with 60 s passive rest periods	Relative to values on a non-exercising control day: 3 hr postprandial GAUC ~↓35%, post meal peak glucose ↓16%, time in hyperglycaemia ↓65%
423	4 men & 7 women, metabolic syndrome, 55±13 years, BMI 30±2, VO_{2max} 34±3	**HIIT**: 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max}	FG ↓~15% below baseline for 72 hr vs CON
	4 men & 4 women, metabolic syndrome, 52±11 years, BMI 29±2, VO_{2max} 36±3	**CME**: 47 min at 70% HR_{max} (matched for energy expenditure with HIIT)	FG ↓~15% below baseline for 24 hr
	5 men & 4 women, metabolic syndrome, 50±9 years, BMI 32±1, VO_{2max} 32±3	**CON**: resting	FG n.s.
506	8 men, healthy, 42±4 years, BMI 29±1, VO_{2max} 53±3	**HIIT**: 4 x 4 min intervals of treadmill running at 85-95% HR_{max} interspersed with 3 min at 50-60% HR_{max} followed by high fat meal 16-18 hours later	N.s. effect on 30 min, 2 hr, and 4 hr postprandial glucose, TAG or HDL between groups.
		CME: 47 min of treadmill walking at 60-70% HR_{max} (to achieve an isocaloric protocol to HIIT) followed by high fat meal 16-18 hours later	
		CON: no exercise followed by high fat meal 16-18 hours later	
505	5 men & 7 women, sedentary or active, 24±3 years, BMI 26±4, VO_{2peak} 38±12	**SIT**: 6 bouts of 30 s cycle sprints at 7.5% BW resistance x 1 session with 4 min active or passive recovery between sprints	72 hours post exercise: FG, insulin & ISI (hyperinsulinemic euglycemic clamp) n.s.

Reference	Baseline Population	Protocol	Metabolism
82	6 men, active, 22±3 years, BMI 25±n.r., & 6 women, active 21±1 years, BMI 22±n.r., VO_{2peak} n.r.	**SIT1**: 30 s cycle sprint at 8.8% BW resistance with 4 min active recovery (no resistance) x 4 in the evening; a high fat mixed meal was consumed after a 13 hour overnight fast	Fasting: glucose, TAG, glucose, insulin & NEFA n.s. from SIT2 & CON; Postprandial AUC vs CON: TAG ↓21%; glucose, insulin & NEFA n.s. Postprandial AUC vs SIT2: TAG ↓12%; glucose, insulin & NEFA n.s.
		SIT2: as above, but the energy used during exercise was replaced immediately post exercise	FG, TAG, insulin & NEFA n.s. from SIT1 & CON; Postprandial AUC vs. CON: TAG ↓10%; glucose, insulin & NEFA n.s.
		CON: no exercise followed by a high fat mixed meal consumed after a 13 hour overnight fast	CON used as comparison group only with no within group baseline vs. post meal comparisons
83	20 men, active, 22±4 years, BMI 23±3, VO_{2max} 53±8	**HIIT**: treadmill running at 115% AT for 3 min intervals with 1.5 min passive rest periods until 500 kcal were expended; high-fat meal 30 min post exercise	Postprandial AUC vs CON: TAG ↓15%, insulin ↓21, VLDL ↓13% Postprandial AUC vs CME:
		CME: treadmill running at 85% AT until 500 kcal were expended; high-fat meal 30 min post exercise	Postprandial AUC vs CON: TAG ↓18%, insulin ↓25%, VLDL n.s.
		CON: no exercise; high-fat meal after 30 min rest	CON used as comparison group only with no within group baseline vs. post meal comparisons
507	9 men, active, 24±3 years, BMI 25±n.r., VO_{2peak} n.r.	**SIT**: 30 s cycle sprint at 7.5% BW resistance with 4 min active recovery (no resistance) x 5 at 1400 hr; see next day meal below	Insulin & glucose response n.s. between groups; TAG AUC ~↓30% v.s. CON
		CME: 30 min brisk (~7 km/h) treadmill walking at 1400 hr; ; see next day meal below	Insulin, glucose, and TAG AUC n.s. vs CON
		CON: 30 min resting at 1400 hr; ; see next day meal below	CON used as comparison group only with no within group baseline vs. post meal comparisons
		Test day: at 0900 the next day a high fat (~59 g fat, ~76 g carbohydrate, 26 g protein) was consumed. An identical meal was consumed at lunch	

Study population demographics (sample size by sex, health or activity description, body mass index in kg/m2, and either VO_{2peak} or VO_{2max} in mL/kg/min as reported by study authors in are provided as means ± standard deviation, where reported, rounded to nearest whole number. Sample sizes are based on those included in the final analysis. Results have been converted to percentage change from baseline if the change was statistically significant followed by indication if this was significant relative to comparison group(s). Results are provided to two significant figures. Where data was reported in graph form it may not have feasible to accurately calculate percentage change so ~ is used to indicate this. The protocol column contains the core exercise but does not describe warm up and cool down protocols, which consisted predominantly of 5-10 minutes periods of light-to-moderated intensity activity. **Abbreviations**: AT, anaerobic threshold; AUC, are under the curve; BMI, body mass index in kg/m^2; BW, body weight; FAS, fatty acid synthase; FG, fasting plasma glucose concentration; h, hour(s), ISI, insulin sensitivity index; MAP, maximal aerobic power; min, minutes; n.r., not reported or data not presented in non numerical formats; s, seconds; TAG, triacylglycerol.

3.3.4.2 Effect of Multiple Bouts

Studies reporting the metabolic effects of HIIT and SIT are summarised in *Table 3.5*.

3.3.4.2.1 Glucose Control and Insulin Sensitivity

Fourteen studies reported fasting glucose (n=179) [422,424,445,446,467,473,474,479,485,488,492,494,505,511], and three noted a reduction post-intervention [422,445,474]. Two further studies noted a reduction in fasting insulin only [485,511]. Studies reporting change generally had greater baseline fasting glucose than those reporting no change. Further, the majority of studies showed a trend toward fasting glucose reduction even when this did not reach statistical significance. The three exceptions were in cohorts with chronic heart failure [446], recent coronary artery bypass grafts [467], and metabolic syndrome [424]. In the case of the first two studies the conditions of the patients severely limited the absolute intensity of the exercise, which may explain the discrepancy in findings. Not finding an effect in metabolic syndrome is less readily explicable. A weight reduction of ~2.5% may have been a contributing factor in the observed change of two studies (*see Table 3.3*) [422,455], but a third study reported reduced fasting glucose without weight change [445] while one study omitted reporting post-intervention weight [511]. Unfortunately, fasting measures are a relatively insensitive to metabolic changes [512,513].

Only seven studies assessed glucose control and/or insulin sensitivity by more rigorous means: five by oral glucose tolerance test (n = 59) [474,479,481,488,494], one by 24-hour continuous glucose monitoring [421], and one by hyperinsulaemic euglycemic clamp [505]. Some studies reported reductions in the area under the insulin curve [479,488], area under the glucose curve [488], improved insulin sensitivity index[479,481,488], or clamp assessed insulin sensitivity [505]. The last study highlighted heterogeneity in response – insulin sensitivity improved in 10 volunteers, remained unchanged in one, and worsened in one [505]. The only study conducted in patients with type 2 diabetes reported reductions in 24-hour and postprandial glucose [421]. However, studies in normal and overweight/obese adults without marked glucose intolerance reported no significant changes in

area under the insulin curve [481], or area under the glucose curve [479,481]. The one study to conduct assessments at multiple time points post-exercise reported changes in area under the insulin curve and insulin sensitivity index at 24 but not 72 hours post-intervention [479]. Given the common practice to ask volunteers to not undertake vigorous physical activity for 48-72 hours prior to metabolic assessment, this may explain discrepancies in study findings.

Six studies compared HIIT/SIT with CMIE [422,445,446,467,474,485], and two with resistance training [424,474]. Overall, the reports suggest HIIT and SIT interventions are comparable or slightly superior to CMIE and conventional resistance exercise for improving markers of glucose control [422,445,474,485].

3.3.4.2.2 Fasting & Postprandial Lipids

Several studies reported pre- and post-intervention fasting lipids: nine triacylglycerol[422,424,445,446,466,468,479,492,494,504], five total cholesterol [445,474,475,494,496], nine high-density lipoprotein cholesterol [424,445,446,466-469,474,475,479,496], and five and low-density lipoprotein cholesterol [445,446,474,475,494]. Only two studies reported any change - an increase in high-density lipoprotein cholesterol [466,468,496]. One study reported reductions in total and low-density lipoprotein cholesterol, and triacylglycerol [494], however this study also employed a calorie-restricted diet, which achieved similar reductions without an HIIT component. Studies comparing HIIT/SIT with CMIE and conventional resistance training indicated that the effects of the latter two were no better. One notable finding was a reduction in circulating very low-density lipoprotein triacylglycerol following eight weeks of HIIT [504]. The observed reduction was shown to relate to reduced hepatic secretion rather than changes in clearance.

3.3.4.2.3 Substrate Utilisation

Four studies reported markers of substrate utilisation following HIIT: two reported no change in respiratory exchange ratio 48 and 72 hours post-exercise, respectively [504,505]; one reported a reduction in respiratory quotient [492]; and one study showed marked increases in fat oxidation and reduction in carbohydrate oxidation 15-60 minutes following exercise [501]. Again, the most

plausible explanation for the differences in findings is the difference in elapsed time between the last bout of exercise and the assessment.

3.3.4.3 Summary of Metabolic Changes following High-Intensity Intermittent and Sprint Interval Training

HIIT and SIT were similarly effective to CMIE in improving glucose control and/or insulin sensitivity in populations where these are impaired, and at a substantially reduced time commitment. HIIT and SIT appear to have little capacity to modify fasting blood lipids. It is unfortunate that none of the studies assessing blood lipids following multiple exercise sessions measured postprandial lipid response as this was shown to be improved in single bout studies. Interestingly, studies reporting improvements with HIIT/SIT also tended to report improvements with CMIE, and *vice versa,* further suggesting that assessment methods and timing at least partly account for inter-study discrepancies.

Future studies and clinical exercise prescriptions should take into account that metabolic changes following exercise can be short-lived. The common practice of asking study volunteers to refrain from vigorous activity for 48-72 hours prior to metabolic assessments may explain negative findings in several small studies. It would seem prudent to assess metabolic response consistent with the frequency of the exercise prescription; e.g. in a thrice weekly intervention with evenly spaced exercise sessions volunteers would, in theory, never go more than 48 hours without exercise. Assessing the ‘chronic’ effects of exercise would best be achieved by measuring longer-term metabolic markers such as HbA_{1c}. Further, postprandial rather than fasting assessments are likely to be both more sensitive and more meaningful.

Table 3.5: Metabolism after Prolonged High-Intensity Intermittent or Sprint Interval Training

Reference	Baseline Population	Protocol	Metabolism
421	8 adults (sex n.r.), type 2 diabetes, 63±8 years, BMI 32±6, VO_{2peak} n.r.	**HIIT**: 10 x 1 min intervals at ~90% HR_{max} with 1 min rest periods 3/wk x 2 wk	CGM (24 hours): mean glucose ↓13%, 24 hr AUGC ↓14%, sum of 3 hr postprandial AUGC ↓30%.
422, 423, 425	4 men & 7 women, metabolic syndrome, 55±13 years, BMI 30±2, VO_{2max} 34±3	**HIIT**: 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 3/wk x 16 wk	IS (from HOMA) ↑24% vs baseline & CME & CON; FG ↓4.3% vs. baseline; TAG n.s.
	4 men & 4 women, metabolic syndrome, 52±11 years, BMI 29±2, VO_{2max} 36±3	**CME**: 47 min at 70% HR_{max} 3/wk for 16 wk (matched for energy expenditure with HIIT)	IS (from HOMA), FG & TAG n.s.
	5 men & 4 women, metabolic syndrome, 50±9 years, BMI 32±1, VO_{2max} 32±3	**CON**: no intervention	IS (from HOMA), TAG & FG n.s.
424	11, metabolic syndrome, 50±10 years, BMI 31±4, VO_{2peak} 34±10	**HIIT**: treadmill walking/running at 90-95% HR_{peak} for 4 min x 4 bouts with active recovery periods of 3 min at 70% HR_{peak} x 3/wk x 12 wk	TAG, HDL & FG n.s.
	11 (sex n.r.), metabolic syndrome, 51±8 years, BMI 32±4, VO_{2peak} 32±5	**RET**: 3 sets of 8-12 repetitions x 3-5 exercises x 3/wk x 12 wk	TAG, HDL & FG, n.s.
	10 (sex n.r.), metabolic syndrome, 53±10 years, BMI 30±4, VO_{2peak} 28±6	**HIIT+RET**: HIIT as above 2/wk, 3 resistance exercises for 8-12 repetitions 1/wk, all for 12 wk	TAG, HDL & FG n.s.
	11 (sex n.r.), metabolic syndrome, 47±10 years, BMI 32±4, VO_{2peak} 34±10	**CON**: no exercise	TAG, HDL & FG n.s.
446	9 men & 5 women, chronic heart failure, 68±6 years, BMI 24±n.r., VO_{2peak} 16±4	**HIIT**: 5 x 3 min cycling intervals at 80% VO_{2peak} with 3 active recovery periods at 40% VO_{2peak} x 3/wk x 12 wk	FG, TC, HDL, LDL, & TAG n.s.
	8 men & 5 women, chronic heart failure, 66±8 years BMI 24±n.r., VO_{2peak} 16±3	**CME**: 30 min cycling at 60% VO_{2peak} x 3/wk x 12 wk	FG, TC, HDL, LDL, & TAG n.s.
	9 men & 4 women, chronic heart failure, 68±9 years, 25±n.r., VO_{2peak} 21±5	**CON**: usual care with advice for home-based physical activity	FG, TC, HDL, LDL, & TAG n.s

Reference	Baseline Population	Protocol	Metabolism
445	8 men, post infarction heart failure, 62±8 years, BMI 28±2, VO_{2peak} 19±5	**HIIT**: 2-4 x 4 min incline treadmill walking at 75-80% HRR with 3 min active recovery at 45-50% HRR 3-5/wk x 12 week (sessions/week:2/2/2/3//3/3/4/4/4/5/5/5)	FG ↓10% vs baseline; insulin, HOMA-IR & TAG n.s. vs baseline but greater reductions than CME; TC, HDL & LDL n.s.
	8 men, post infarction heart failure, 63±9 years, BMI 27±3, VO_{2peak} 18±4	**CME**: 30-45 min incline treadmill walking at 45-60% HRR 3-5/wk x 12 week (sessions/week:2/2/2/3//3/3/4/4/4/5/5/5) with variables matched to the HIIT group week by week	TC ↓6%; FG, insulin, HOMA-IR, HDL, LDL, & TAG n.s.
466, 467	25 men & 5 women, post myocardial infraction, 57±10 years, BMI 27±3, VO_{2peak} 32±6	**HIIT:** 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 3/wk x 12 wk	HDL ↑3% vs baseline; TAG n.s. At 6 months: HDL declined to baseline level; TAG n.s.
	49 men & 10 women, post myocardial infraction, 58±9 years, BMI 27±4, VO_{2peak} 32±7	**Group session:** 10 min warmup, 35 min aerobic exercise (walking/jogging/lunges), 15 min cooldown, stretching & relaxation.	HDL & TAG n.s. At 6 months: HDL & TAG n.s.
468, 469	24 men & 4 women, following CABG, 60±7 years, BMI 26±6, VO_{2peak} 27±5	**HIIT:** 4 x 4 min incline treadmill walking at 90-95% HR_{max} with 3 min recovery periods at 70% HR_{max} 5/wk x 4 wk supervised, then advised to continue at home	At 4 wk: HDL & glucose n.s. At 6 months: HDL & glucose n.s.
	24 men & 7 women, following CABG, 62±8 years, BMI 28±4, VO_{2peak} 26±5	**CME:** 47 min at 70% HRmax 5/wk for 4 wk (matched for energy expenditure with HIIT), then advised to continue at home	At 4 wk: HDL & glucose n.s. At 6 months: glucose & HDL n.s.
479	10 men, sedentary overweight/obese, 32±9 years, BMI 31±4, VO_{2peak} 33±1	**SIT**: 4-7 bouts of 30 s cycle sprints at 6.5% FFM resistance 3/wk for 2 wk (sprints/session: 4/4/5/5/6/6) with 4.5 min active at 30 W resistance	AUIC ↓15% & ISI ↑23% 24 hr post last session but n.s. 72 hr post; AUGC, TAG, HDL, FG, fasting insulin all n.s. at 24 & 72 hours post last session

Reference	Baseline Population	Protocol	Metabolism
474	7 (sex n.r.), sedentary overweight, 41±12 years, BMI 31±3, VO_{2peak} ~23±n.r.%	**HIIT**: 10 x 1 min cycling at 90% VO_{2peak} with 2 min recovery at 30% VO_{2peak} x 4 sessions/wk for 8 wk, & diet education (1 hour seminar)	TC, LDL-C & HDL-C n.s.
	6 (sex n.r.), sedentary overweight, 45±17 years, BMI 30±3, VO_{2peak} ~24±n.r.%	**CME**: 50% VO_{2peak} matched to energy expenditure of HIIT for time, & diet education (1 hour seminar).	TC, LDL-C & HDL-C n.s.
	8 (sex n.r.)., sedentary overweight, 40±13 years, BMI VO_{2peak} ~23±n.r.%	**Diet**: diet education only (1 hour seminar).	TC, LDL-C & HDL-C n.s.
494	2 men & 8 women, obese, 37±10 years, BMI 32±4, VO_{2peak} 27±5	**HIIT+Diet:** Up to 10 x 4 minutes cycling at 90% $VO2_{peak}$ with 2-3 minutes rest periods, for 6 supervised sessions over 14 days. (see below for diet)	TC ↓7.8%, LDL ↓7.1%, TAG ↓27% vs baseline; FG, fasting insulin, 2 hr glucose, & AUGC n.s.
	3 men & 6 women, obese, 41±14 years, BMI 32±3, VO_{2peak} 26±9	**Diet**: 75% of food record estimated requirement (35% CHO)	TC ↓10%, LDL ↓7.6%, TAG ↓27% vs baseline; FG, fasting insulin, 2 hr glucose, & AUGC n.s.
473	12 men, overweight/obese, 24±5 years, BMI 29±3, VO_{2peak} 38±6	**HIIT:** 6 x 4 min cycling at 85% VO_{2peak}, with 2 min rest x 6 sessions over 14 d.	FG, & fasting insulin n.s.
505	2 men & 7 women, sedentary or active, 29±9 years, BMI 26±4, VO_{2peak} 33±6	**SIT**: 4-7 bouts of 30 s cycle sprints at 7.5% BW resistance 3/wk for 2 wk (sprints/session: 4/5/6/6/7/4) with 4 min active or passive recovery between sprints	IS (from hyperinsulinemic euglycemic clamp) ↑~20% (72 hours post last exercise session); RER n.s. vs. CON; FG, insulin & NEFA n.s.
	2 men & 8 women, sedentary or active, 23±3 years, BMI 28±5, VO_{2peak} 35±9	**CON**: no exercise	IS (from hyperinsulinemic euglycemic clamp); RER, FG, insulin & NEFA n.s.
496	20 men, active, 30±5 years, BMI 23±4, VO_{2peak} n.r.	**HIIT**: 4 x 800 m runs at ~90% age predicted HR_{max} (220-age) with a 1:1 work-to-rest ratio 3/wk x 8 wk	HDL ↑18%, TC/HDL ↓18% vs baseline & CON; TC n.s.
	16 men, active, 30±5 years, BMI 23±2, VO_{2peak} n.r.	**CON**: no vigorous exercise	TC, HDL & TC/HDL n.s.

Reference	Baseline Population	Protocol	Metabolism
511	4 men & 3 women, sedentary, 45±5 years, BMI 27±5, VO_{2peak} 30±3	**HIIT:** 1 min intervals at 60% W_{max} with 1 min active recovery at 30 W resistance x 10 intervals x 3/wk x 2 wk	72 hr post last exercise: insulin ↓16%; HOMA-IR ↓35%; glucose n.s.
492	20 men, sedentary & overweight, 25±5 years, BMI 28±1, VO_{2peak} 34±1	**SIT:** 8 s sprints with 12 s recovery periods at a cadence of 120-130 & 40 RPM, respectively, for 20 min at 80-90% HR_{peak} x 3/wk for 12 w	RQ ↓2.4% vs. CON; glucose, HOMA-IR, TAG & TC n.s.
	18 men, sedentary & overweigh, 25±4 years, BMI 29±1, VO_{2peak} 29±1	**CON:** no exercise	Only used as a comparator for SIT
474	8 men, sedentary, 37±8 years, BMI n.r. (BW 96±11 kg), VO_{2peak} 36±5	**HIIT**: 5 x 2 min running >95% HR_{max} with 2 min rest periods 3/wk x 12 wk	FG↓8.8% & 2-hr glucose ↓16% (OGTT); fasting insulin, TC, LDL, & HDL n.s.
	9 men, sedentary, 31±6 years, BMI n.r. (86±16 kg), VO_{2peak} 39±7	**CME**: 60 min running at 80% HR_{max} (~65% VO_{2peak}) 3/wk x 12 wk	FG ↓8.8% & 2-hr glucose ↓13% (OGTT) vs baseline; TC, LDL-C, & HDL-C n.s.
	8 men, sedentary, 36±6 years, BMI n.r. (95±24 kg), VO_{2peak} 38±9	**RET**: 60 min 3-4 sets of: squat, hack squat, incline leg pres, isolated knee extension, hamstring curls, and calf raises 3/wk x 12 wk	TC↑10%; FG, 2-hr glucose (OGTT), fasting insulin, LDL-C, & HDL-C n.s.
	11 men, sedentary, 30±6 years, BMI n.r. (87±11 kg), VO_{2peak} 39±8	**CON**: no exercise x 12 wk	FG, 2-hr glucose (OGTT), fasting insulin, TC, LDL-C, & HDL-C n.s.
481	8 women, sedentary, 23±3 years, BMI 23±2, VO_{2peak} 33±2	**SIT:** cycling 10 min total containing 2 all out sprints for 10/15/20/20/20/20 s in wk 1-6 x 3/wk, respectively, remaining time was at 60 W	Glucose AUC ↑6% vs baseline but n.s. vs CON; insulin AUC (OGGT), & ISI (Cederholm) n.s.
	8 women, sedentary, 22±1 years, BMI 23±2, VO_{2peak} 33±2	**CON:** no exercise	Glucose AUC ↑14% vs baseline; insulin AUC (OGGT), & ISI (Cederholm) n.s. vs baseline or CON

Reference	Baseline Population	Protocol	Metabolism
481	7 men, sedentary, 26±3 years, BMI 24±2, VO_{2peak} 36±2	**SIT:** cycling 10 min total containing 2 all out sprints for 10/15/20/20/20/20 s in wk 1-6 x 3/wk, respectively, remaining time was at 60 W	ISI (Cederholm) ↑28%; glucose & insulin AUC (OGGT) n.s. vs baseline or CON
	6 men, sedentary, 25±2 years, BMI 24±2, VO_{2peak} 38±3	**CON:** no exercise	glucose & insulin AUC (OGGT), & ISI (Cederholm) n.s.
501	10 women, active, 22±1 years, BMI n.r. (BW 65±2 kg), VO_{2peak} ~43±n.r.	**HIIT**: 10 x 4 min cycling at 90% VO_{2peak} with 2 min active rest periods 3/wk x 6 wk	Baseline vs wk 2 15-60 min post exercise: FA oxidation ↑64%, CHO ↓18% oxidation. Baseline vs wk 6 15-60 min post exercise: FA oxidation ↑68%, CHO ↓21% oxidation.
488	16 men, active, 22±2 years, BMI 24±3, VO_{2peak} 48±9	**SIT**: 4-6 30 s cycle sprints against resistance of 7.5% BW with 4 min unloaded cycling between sprints x 3/wk x 2 wk (sprints per session: 4/4/5/5/6/6)	Glucose AUC ↓12%, insulin AUC ↓37%, IS (Cederholm index) ↑23%, NEFA AUC ↓26%
485	15 healthy women, 22±1 years, BMI 24±2, VO_{2peak} 29±8	**SIT**: 3 sessions/wk over 15 weeks: 8 s sprints/12 s active (20-30 rpm) recovery at 0.5 kg resistance. Progressing from 5 min sessions to 20 min sessions with 0.5 kg resistance added once 20 minutes	HOMA-IR ↓33% vs baseline but n.s. vs. CME & CON; fasting insulin ↓31% vs baseline & CON
	15 healthy women, 21±1 years, BMI 22±1, VO_{2peak} 31±8	**CME**: 3 sessions/wk over 15 weeks: 10-20 min at 60% VO_{2peak} for 10-20 min progressing to 40 minutes	HOMA-IR ↓11% but n.s. vs CON, fasting insulin ↓9% vs baseline but n.s. vs CON
	15 healthy women, 22±1 years, BMI 24±1, VO_{2peak} 31±6	**CON**: no change in physical activity	HOMA-IR n.s. vs baseline, fasting insulin n.s. vs baseline
495	7 men, active, 20-40 years, BMI 25±3, VO_{2peak} 37±7	**HIIT**: 4 x 4 min treadmill running at 90% VO_{2peak} with min recovery periods at 60% VO_{2peak} 3/wk x 8 wk	48 hr post last session RER, NEFA, TAG n.s.; VLDL-TAG ↓28% vs baseline & CON
	8 men, active, 20-40 years, BMI 23±2, VO_{2peak} 40±16	**CON**: no intervention	RER, NEFA, TAG & VLDL-TAG n.s.

Study population demographics (sample size by sex, health or activity description, body mass index in kg/m2, and either VO_{2peak} or VO_{2max} in mL/kg/min as reported by study authors in are provided as means ± standard deviation, where reported, rounded to nearest whole number. Sample sizes are based on those included in the final analysis. Results have been converted to percentage change from baseline if the change was statistically significant followed by indication if this was significant relative to comparison group(s). Results are rounded to two significant figures. Where data was reported in graph form it may not have feasible to accurately calculate percentage change so ~ is used to indicate this. The protocol column contains the core exercise but does not describe warm up and cool down protocols, which consisted predominantly of 5-10 minutes periods of light-to-moderated intensity activity. **Abbreviations**: AUC, area under the curve; BMI, body mass index (kg/m2); BW, body weight; CABG, coronary artery bypass graft; CGM, continuous glucose monitoring; CON, control group; FG, fasting plasma glucose concentration; hr, hour or hours; HRR, heart rate reserve; HDL, high-density lipoprotein; HIIT, high intensity intermittent training group; IS, insulin sensitivity; ISI; insulin sensitivity index; min, minutes or minutes; NEFA, non-esterified fatty acids; n.r., not reported; n.s., no statistically significant change/difference; OGTT, oral glucose tolerance test; OGIS, insulin sensitivity calculated from 2-hour oral glucose tolerance test; s, seconds; RER, respiratory exchange ratio; RET, resistance exercise training group; RM, repetition maximum; RPE, rate of perceived exertion; RPM, revolutions per minute; RQ, respiratory quotient; SIT, sprint interval training group; TAG, triacylglycerol; TC, total cholesterol; VLDL, very low-density lipoprotein; W_{max}, maximum workload.

3.3.5 Study Protocols and Study Quality

A broad range of exercise protocols were used (see *Tables 3.1 - 3.5*). The duration of multi-session interventions was between 2 and 26 weeks with the single most common duration being 12 weeks. Session frequency was ranged between 1.5 and 5 times per week, but was usually three times per week with only 5 studies exceeding this. The criteria for HIIT interventions are provided in *Section 3.2.3* above. Most protocols accumulated between 10 and 16 minutes at high intensity using either cycle ergometers or treadmills; consistent with what is generally available in an exercise laboratory setting. SIT in healthy populations commonly involved some variant of the Wingate protocol: 30 second *all out* efforts against a resistance of 5-10% of an individuals body weight with either passive or low intensity active rest. SIT was also used in cardiac rehabilitation settings, but in this scenario submaximal rather than supramaximal efforts were required, i.e. although interval length met the proposed definition of SIT in *Section 3.2.3*, the intensity of the effort was consistent with the definition for HIIT.

In the studies reviewed, sample sizes tended to be low and exercise sessions were either always or frequently supervised. This is suited to establishing efficacy and may even reflect effectiveness in cardiac rehabilitation and similar short-term supervised approaches to clinical exercise, but it is a poor model for assessing the likely effectiveness of such interventions in the community where cost prohibits close supervision of the many people with NAFLD and related conditions. Thus most if not all these trials are essentially phase 1 or phase 2 in nature, and their protocols remain to be tested in trials designed to assess effectiveness in a scenario more closely reflecting the clinical situation.

3.4 Summary and Conclusion

The studies reviewed suggest that, provided undiagnosed and unstable cardiovascular disease is ruled out, HIIT has a good safety profile and is well tolerated in a range of healthy and clinical populations relevant to NAFLD. Indeed the limited number of reports assessing patient preference indicate many would prefer an intermittent approach to their exercise sessions and, despite greater intensities being reached, perceive such an approach as overall less hard than the same amount of work performed in a continuous fashion. The safety of SIT using supramaximal aerobic intensities have not been tested in groups with or at high risk of heart disease, so it is prudent not to use this approach in clinical populations as present. Further, SIT may simply be too hard for some deconditioned patients to perform as intended, although this is not clearly established.

HIIT and SIT at frequencies of three or more times per week and a total time or 20-40 minutes per session, have consistently been reported to result in substantial and rapid improvements in cardiorespiratory fitness and work capacity. These improvements have generally exceeded those observed in CMIE groups when matched for total work, and been similar even when the total work performed for HIIT or SIT was well below that performed by CMIE groups. Although not as consistent across studies, reports in patients with or at elevated risk of hypertension showed a reduction in blood pressure and improvements in other haemodynamic responses following single and multiple bouts of HIIT. Given the inverse association of physical activity and cardiorespiratory fitness and intrahepatic fat [232,233,238,239], the strong tendency toward sedentariness in those with NAFLD [237], and the inverse association between physical activity and disease progression [208,240], HIIT presents a plausible and time efficient means by which to reduce intrahepatic fat and risk of disease progression.

Studies to date showed a tendency toward modest weight reduction and, more consistently, positive changes in body composition following 12 or more weeks of HIIT. Further, single bouts of HIIT improved glucose control in patients with type 2 diabetes and metabolic syndrome and even healthy young volunteers,

but in the latter case only when exercise related energy expenditure was high. Single bouts of both HIIT and SIT also reduced postprandial lipid response. The majority of studies in populations with impaired glucose tolerance/insulin resistance also reported improvements in glucose control and/or insulin sensitivity following multiple sessions of HIIT and SIT, whereas studies in normoglycaemic populations were equivocal. A likely confounding factor was the heterogeneity of time between last bout of exercise and measurements being made. Further, despite acute changes to postprandial lipid metabolism, fasting lipids commonly assessed in clinical practice appeared largely unaffected by HIIT or SIT. Overall, changes to metabolism were generally only detectable 24-48 hours following exercise suggesting a frequency of roughly every other day is appropriate and tolerable. Of note is that HIIT and SIT induced changes tended to last longer than those following CMIE.

The principle of HIIT, although most frequently tested on cycle ergometers and treadmills, can easily be applied to a range of equipment available to patients in their homes or at commercial fitness facilities. It is also feasible to apply HIIT approaches to outdoor walking/running/jogging or cycling. Although not formally assessed, the use of cycle ergometers most likely provides the safest option for many patients as there is minimal joint impact, low likelihood of falling, and it can take place in a safe controlled environment. Although most studies employed supervised exercise with prescriptions based on preset parameters such as cycle resistance or treadmill speed and incline, using self-assessed rating of perceived exertion was equally effective. Such self-guided intensity is much more applicable in the clinical context.

Thus HIIT has a good potential to be a safe and well tolerated, and above all time efficient means by which to improve fitness, metabolic health, and potentially body composition in patients with NAFLD.

Chapter 4. The Physiological Effect of Age and Activity on Metabolism Study

4.1 Introduction

The prevalence of metabolic conditions such as non-alcoholic fatty liver disease (NAFLD) [5], metabolic syndrome [514,515], and type 2 diabetes increases with age [516,517]. Similarly, the prevalence of metabolic diseases and the risk of all-cause mortality increases with increasing sedentary time and decreasing physical activity [518]. Further, low cardiorespiratory fitness, independent of BMI, is associated with increased general morbidity and mortality, particularly in relation to metabolic diseases and cardiovascular conditions [519-523], and also cancer [524]. There is an inverse association of cardiorespiratory and muscular fitness with parameters such as intrahepatic triacylglycerol (IHTAG) concentration [8,239], insulin resistance [519], and all cause mortality [525-529]. The association of better physical fitness with better long-term health prospects was still evident among populations already with impaired fasting glucose [527], type 2 diabetes [527,530], and pre-existing cardiovascular disease [526,531,532]. This is still evident among the those aged over 80 years [533].

Age and physical activity are generally closely and inversely associated [534], which in turn explains at least some of the association between age and metabolic conditions. Thus there is a need to identify to what extent the unmodifiable risk factor of chronological age and the readily modifiable risk factor of inactivity account for shifts in disease prevalence and severity. And a further need to identify to what extent cardiorespiratory fitness influences this relationship.

To address these questions, cross-sectional data were collected from three age groups of women 20-30 years (young), 40-50 years (middle-aged), and 65 years and over (older). Data were subdivided into those from sedentary and active volunteers. Step count was averaged over seven days; volunteers with a daily step count under 7500 were admitted to the study as the sedentary group and those exceeding 12500 were admitted as the active group.

The aims of this analysis were to:

- Define the relationship between age and:
  - IHTAG
  - Glucose control
  - Insulin sensitivity (HOMA-IR)
  - Total body fat and fat distribution (abdominal visceral and subcutaneous fat)

- Define the modifying effect of physical activity category on:
  - IHTAG
  - Glucose control
  - Insulin sensitivity (HOMA-IR)
  - Total body fat and fat distribution (abdominal visceral and subcutaneous fat)

- Assess the modifying effect of cardiorespiratory fitness and body composition on the above relationships.

4.2 Methods

This was a case-control study aiming to enrol 24 age-matched active (n=12) and sedentary (n=12) adult females in the following age categories: 20-30 years (young); 40-50 years (middle-aged); and over 60 years (older) for a total n=72.

Objectively assessed 7-day average step-count: ≥12500 steps/day was classified as active; over 7500 steps/day was considered sedentary. Those with step counts between 7500 and 12500 were excluded (see *Section 4.2.1* for details). Women who did regular pre-planned exercise were excluded in line with a desire to determine the effects of general physical activity. Additional exclusion criteria were: pregnancy; implanted ferrous material; conditions or medication precluding maximal exercise testing; mental illness; and chronic disease.

The study was given ethical approval by the Newcastle and Northeast Tyneside Local Research Ethics Committees. All volunteers were provided with a participant information sheet (see *Appendix 2)* before providing written informed consent (see *Appendix 3*).

Volunteers were recruited from the Northeast of England by advertising on physical and electronic notice boards, and word of mouth.

4.2.1 Physical Activity Screening and Monitoring

Step count was measured by the SenseWear BMS multisensor array (BodyMedia Inc., Pittsburgh, PA, USA). Armbands were worn at the midline of the triceps of the right arm for seven days (see *Figure 4.1*).

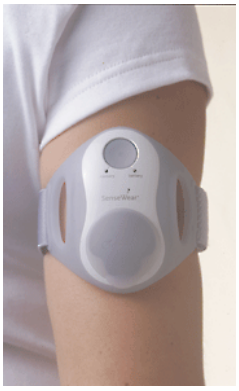


Figure 4.1: SenseWear BMS multisensor array for as worn by study volunteers.

4.2.2 Medical History and Physical Examination

Volunteers were asked to complete the American College of Sports Medicine (ACSM) Physical Activity Readiness Questionnaire (*Appendix 4*), and underwent a physical examination comprising of: anthropometry including height, weight, and waist and hip circumference; auscultation of the heart and lungs; evaluation of the abdomen for any abnormalities; inspection of the lower extremities for oedema and arterial pulses; assessment of reflexes; resting 12 lead electrocardiogram (ECG) (Custo med GmbH, Ottobrunn, Germany) and resting blood pressure (Suntech Tango+, Suntech Medical Ltd, Oxford). These determined if the volunteer was suitable for maximal progressive exercise testing according to the guidelines of the American College of Sports Medicine [436].

4.2.3 Maximal Progressive Exercise Test

Exercise testing was carried out on a Corival Recumbent cycle ergometer (Lode BV, Groningen, the Netherlands). Cardiac rhythm was measured throughout, and blood pressure every three minutes and at peak capacity; with the equipment noted above. Respiratory parameters including peak oxygen uptake (VO_{2peak}) and respiratory exchange ratio were measured by a Metalyzer (CORTEX Biophysik GmbH, Leipzig, Germany). The device was calibrated daily for gas volume and composition, and before each test for ambient air pressure.

Volunteers were asked to sit quietly on the ergometer for 2-3 minutes to obtain resting measures and calibrate equipment. Thereafter the exercise test proceeded with a 5-minute warmup at 25 W resistance before an increase to 50 W followed by 10 W/minute increase in resistance until the participant could no longer maintain a cadence ≥60 rpm, chose to stop, or a contraindication to continuing arose (see of *Appendix 5* for contraindications to exercise).

4.2.4 Magnetic Resonance Imaging

All magnetic resonance imaging and spectroscopy was done using a 3T Philips Achieva scanner (Philips, Netherlands).

4.2.4.1 Liver Fat Magnetic Resonance Imaging

IHTAG was assessed by three-point-Dixon analysis. Three gradient echo scans were acquired with adjacent out-of-phase (OP), in-phase (IP) and out-of phase echoes. (TR/TE/NSA/FA=50/3.45,4.60,5.75/1/30, matrix: 160x109, median FOV 440 mm, range 400-480 mm to suit subject size with 70% phase FOV), bandwidth 2.7 kHz/pixel. Sets of 6 slices were acquired within a 16 second breath-hold. Three contiguous blocks of 6 slices were acquired in three separate breath-holds to cover the largest cross-section of liver with 10 mm thick slices. Correction was made from the first and third echoes for T2* relaxation, and from prior knowledge for T1-weighted correction. Images were analysed using a custom Matlab script, and registered using FSL tools (University of Oxford, United Kingdom) before region of interest analysis using the polygon function of ImageJ. The average of 5 slices was taken.

4.2.4.2 Abdominal Visceral and Subcutaneous Fat Determination

Abdominal visceral and subcutaneous fat were measured at L4/L5 using a three-point Dixon sequence (TR/TE/number of averages/flip angle 50 ms/ 3.45, 4.60, 5.75 ms/1/308, matrix 160x109, median field of view (FOV) 440 mm, range 400-480 mm to suit subject size with 70% phase FOV). The slice was acquired during a breath-hold and with slice thickness of 10 mm [535,536]. Fat and water were separated, and binary gating applied to produce a map of structures containing more than 50% fat. ImageJ (National Institute of Health, USA) was

used to calculate total, subcutaneous, and visceral fat area by creating a binary image and using a watershed algorithm [537].

4.2.5 Blood Collection and Analysis

Blood was collected by venipuncture (BD Vacutainer Safety-Lok™, BD Diagnostics, Plymouth, UK) of an antecubital vein into BD K3E and SST™ advance vacutainers (BD Diagnostics, Plymouth, UK) after a 10-12 hour overnight fast. Fasting samples were analysed for: total and high-density lipoprotein (HDL)cholesterol; triacylglycerols (TAG); aspartate aminotransferase (AST); alanine aminotransferase (ALT); γ-glutamyltransferase (GGT); and haemoglobin A_{1c} (HbA_{1c}). Except for whole blood glucose, plasma insulin, and serum NEFA concentrations (see *Section 4.2.6*), all blood sample analyses were carried out in Clinical Pathology Accredited (CPA) laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry).

Total cholesterol [538], HDL-cholesterol [539], ALT [540], and AST [541] were measured using the Roche Modular P and test kits (Roche Diagnostics Ltd, Burgess Hill, UK). LDL-cholesterol was calculated using the the Friedewald equation [542]. GGT was measured using a Roche Modular E170 [543]. HbA1c was measured using a TOSOH HLC-723G7 (Tosoh Corporation, Tokyo, Japan). Serum ferritin was measured by radioimmunoassay [544].

4.2.6 Oral Glucose Tolerance Test

Fasting and blood collection was as described above. Participants were given a 394 mL Lucozade Original glucose drink (GlaxoSmithKline, Gloucestershire, UK), corresponding to 75 g of glucose, to consume within three minutes. Blood samples were taken at time 0 and 120 minutes.

Whole blood glucose was collected into fluoride oxalate tubes (Teklab, Sacriston, UK) and measured immediately with a YSI 2300 Stat Plus-D (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Additional whole blood was collected in K-EDTA tubes (Teklab, Sacriston, UK) and centrifuged at 3000 rpm

(2133 RCF) for 10 minutes in a Sigma 6K15 laboratory centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany).
Fasting plasma insulin was measured by the hospital laboratory described above.

Insulin sensitivity was assessed by homeostatic model assessment of insulin resistance (HOMA-IR) [545]:

$$\text{HOMA-IR} = \text{FI} \times \text{fasting glucose concentration} / 22.5$$

where FI is fasting insulin concentration in mU/mL and FG is fasting glucose concentration in mmol/L.

4.2.7 Body Composition and Anthropometry

4.2.7.1 Air Displacement Plethysmography

Patient body composition, in terms of total body weight, fat mass (FM), fat free mass (FFM), and percentage of body fat, was measured by air displacement plethysmography using a BodPod (Life Measurement Inc., Concord, CA, USA). Participants were asked to wear minimal and/or tight fitting clothing, and a swimming cap, to minimise the potential for air trapped in clothing or hair to affect the measurement. Scales were calibrated weekly.

4.2.7.2 Anthropometry

Height was measured using a stadiometer (SECA 799; SECA, Birmingham, UK). Weight was measured using the scales of the BodPod described above. Waist and hip circumference were measured at the widest point between the lower costal margin and the superior iliac crest, and the widest point around the buttocks, respectively, using a non-stretch tape measure.

4.2.8 Statistics

Statistical analysis was performed using Microsoft Excel 2008 for Mac (Microsoft Corporation, Silicon Valley, USA) descriptive statistics and SPSS 19

(IBM, New York, USA) for all other analyses. Three sets of analyses were performed:

1) comparison by age category only;
2) comparison by age and physical activity categories; and
3) correlations including the full dataset

Skewness and kurtosis for each parameter of interest were assessed by dividing each by its respective standard error to assess the likelihood of a given variable not being normally distributed. Variables for which both skewness and excess kurtosis were between 2 and -2 were analysed without adjustment, whereas those above 2 or below -2 were transformed by the natural logarithm to improve the normality of distributions where parametric tests were used.

Analyses of variance (ANOVA) were performed for all variables of interest and Tukey's honest significance test was performed where the ANOVA indicated a statistically significant difference existed between groups. Analyses of covariance (ANCOVA) were also performed for comparisons done by age and physical activity:

1) adjusting for cardiorespiratory fitness as assessed by VO_{2peak} calculated relative to fat free mass ($VO_{2peakFFM}$) to try and differentiate between the effects of physical activity and cardiorespiratory fitness; and
2) adjusting for body composition as assessed by body fat percentage to assess if differences were strongly mediated by body composition.

$VO_{2peakFFM}$ was used in preference to VO_{2peak} per kg body weight to reduce confounding from the marked differences in body composition between some groups.

Only those comparisons where ANOVA showed significant differences between groups and where the adjustment was not for a clearly related parameter to the one being assessed were subjected to ANCOVA. Thus for the first ANCOVA comparisons of BMI, body fat percentage, fasting glucose, 2-hour glucose, IHTAG, abdominal subcutaneous fat area, and abdominal visceral fat area were adjusted for $VO_{2peakFFM}$. And for the second ANCOVA comparisons of fasting

glucose, 2-hour glucose, IHTAG, and abdominal visceral fat were adjusted for body fat percentage.

Spearman's rank correlations were used to further examine the relationships between indicators of cardiorespiratory fitness, physical activity, body composition, glucose control, and age. This technique is relatively robust even when correlating non-normally distributed variables, therefore none of the variables were transformed irrespective of distribution. Because HOMA-IR is a composite of glucose and insulin no correlations were calculated for this variable.

4.3 Results

A total of 61 women were included in the analysis. The participant flow and reasons for exclusion are presented in *Figure 4.2.* Comparisons between groups by age are shown in *Table 4.3.1*, those by age and physical activity level in *Table 4.3.2.*, and correlations in *Table 4.3.3*. *Figure 4.2* shows the participant flow.

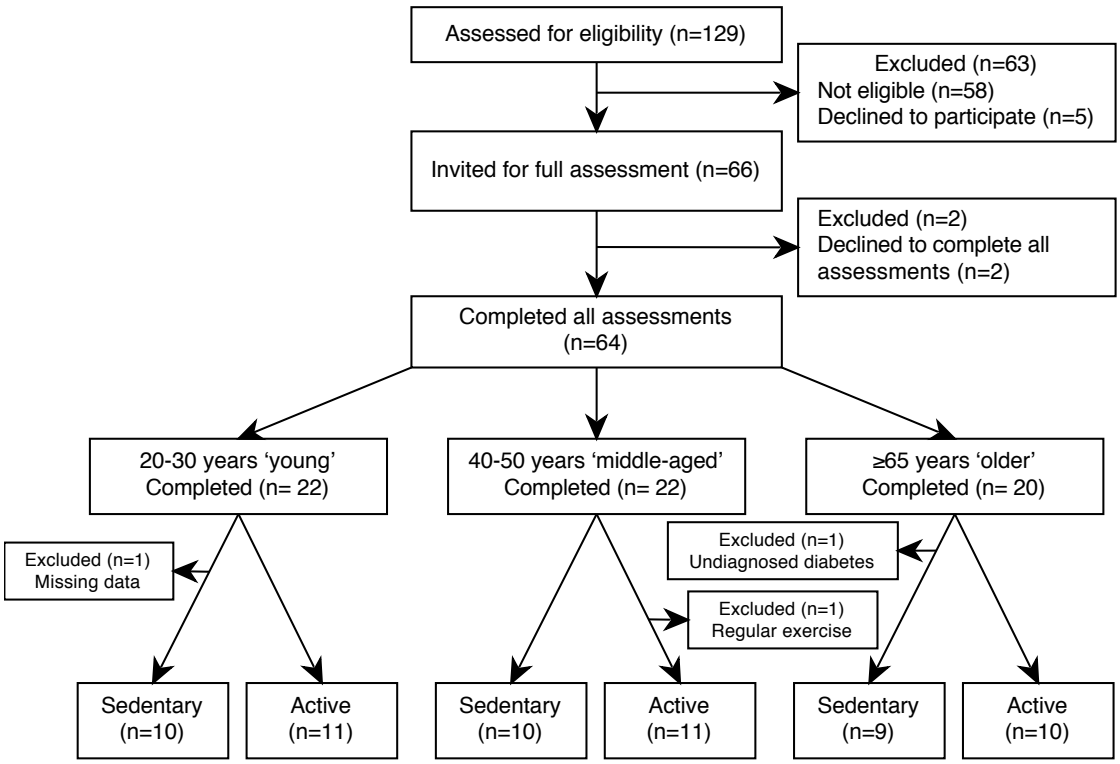


Figure 4.2: Diagram showing participant flow.

The original protocol defined volunteers with a daily step count ≤7500 as sedentary and those with a daily step count ≥12500 as active with those in between excluded from the study. It proved extremely difficult to recruit women aged 20-30 years with daily steps counts <7500, so six volunteers with steps counts >7500 (range: 7710-10778) were included in the sedentary group. For much the same reason four women were included in the active older group despite not quite meeting the ≥12500 step count criteria (range of counts for those four: 11323-11839). In the 40-50 year old group only two volunteers in the active group had step counts below the predefined criteria (counts: 10828 and 11106). Despite these variations, when groups were pooled by age there was no statistically significant difference between groups in step count. When

groups were further subdivided by age and physical activity category – sedentary and active – there were no significant differences in mean step counts between women in the same physical activity category across the three age categories. Scatter plots by age (see *Figure 4.*2), and by age and physical activity category (see *Figure 4.3*), suggest a tendency for sedentary young women in this cohort to be more active than sedentary middle-aged or older women.

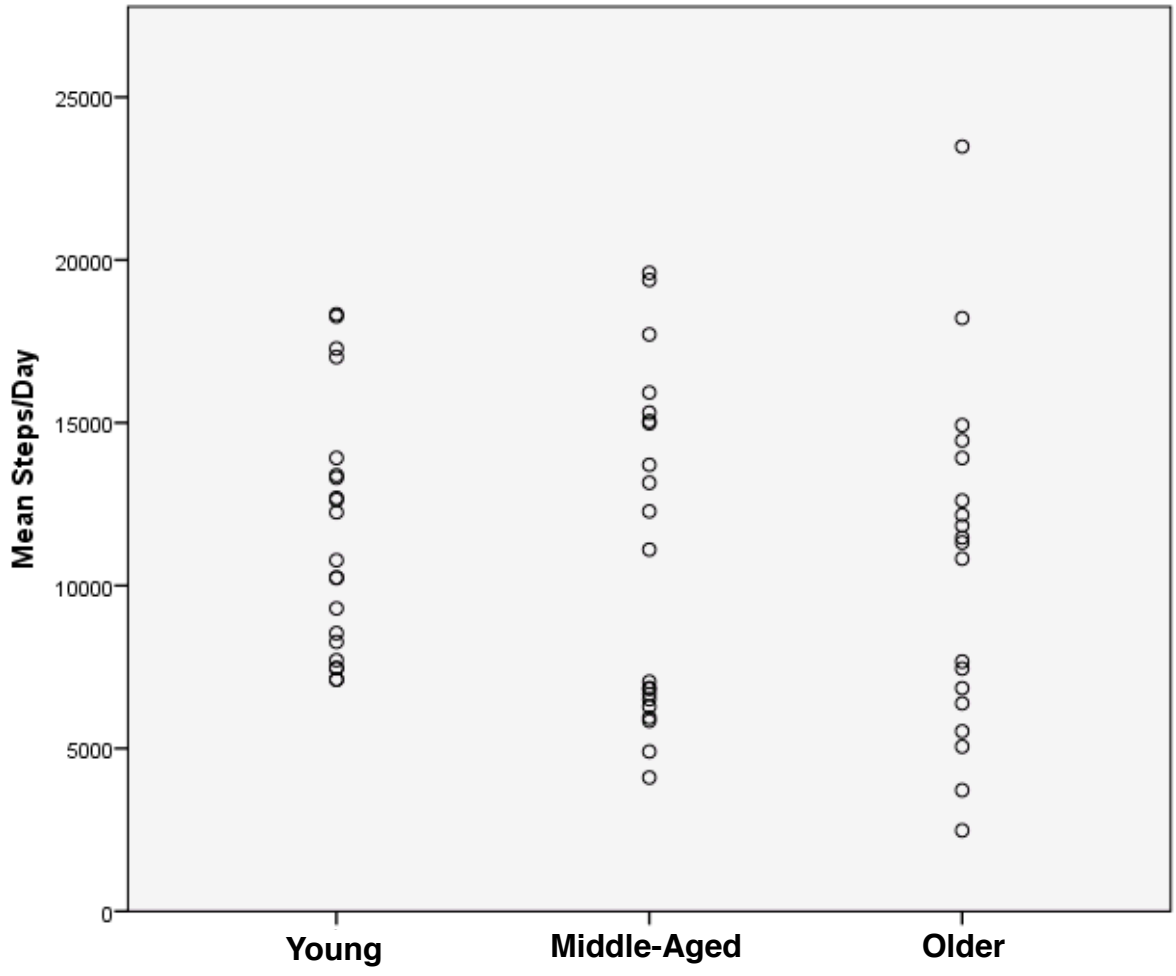


Figure 4.3: Scatter plots of average daily step count distribution by age category.

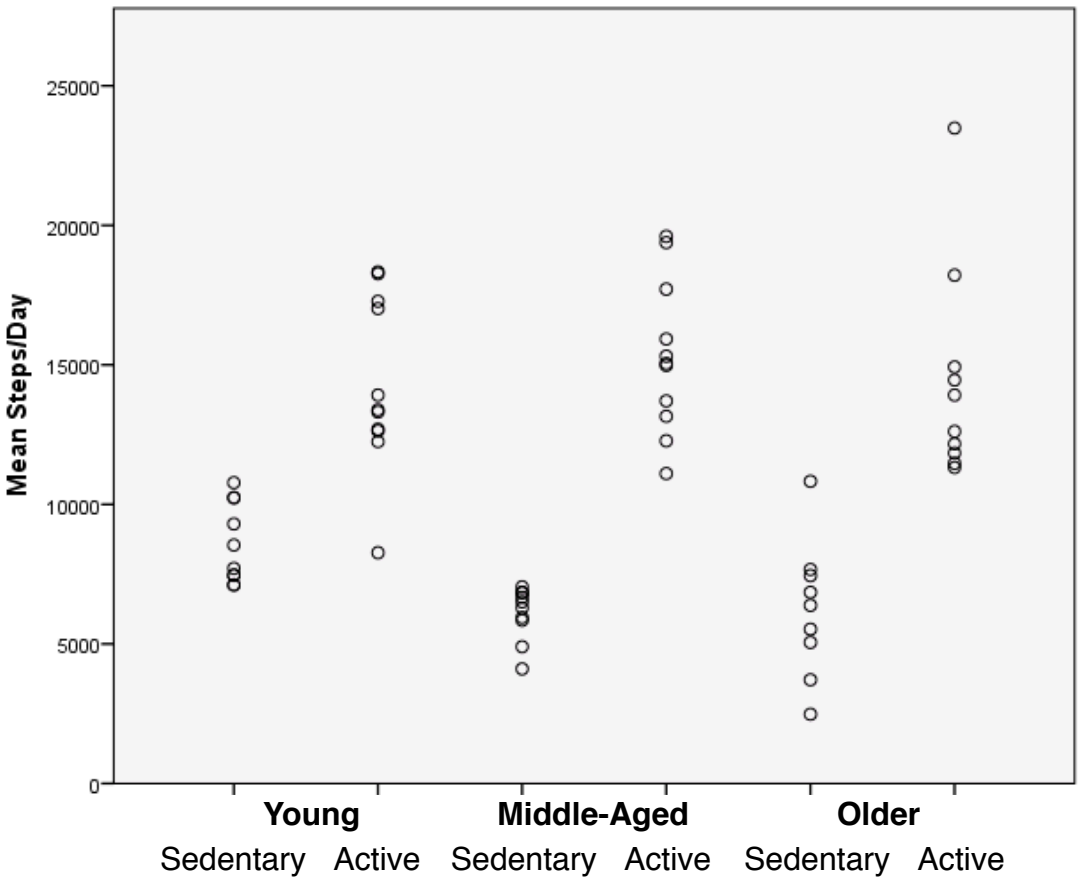


Figure 4.4: Scatter plots of average daily step count distribution by age and physical activity category.

By design, the three age groups were significantly different in terms of age. However, within each age category sedentary and active women were not significantly different.

4.3.1 Body Composition and Liver Fat by Age

Results by age category are shown in *Table 4.1.* The three groups had similar BMI, body fat percentage, and abdominal subcutaneous adipose area. However, body fat was weakly correlated with age ($r = 0.274$, $p \leq 0.05$). Visceral fat area was significantly lower in young and middle-aged relative to older women, and was positively correlated with age ($r = 0.409$, $p = 0.01$).

IHTAG concentration was lower in young than older women, notably so in active young women relative to sedentary older women. The difference between middle-aged and older women suggested an increase with age but did not reach significance (2.04% vs 3.88%, $p = 0.067$). The correlation between

IHTAG and age also did not reach significance but again suggested a weak relationship ($r = 0.246$, $p = 0.056$) (*Table 4.3*). However, fasting insulin and glucose were correlated with IHTAG ($r = 372$, $p \leq 0.01$; and $r = 330$ $p \leq 0.05$, respectively.

Table 4.1: Volunteer Comparison by Age

	Young (n of 21)	Middle (n of 21)	Older (n of 19)	young vs. middle *p*	young vs. older *p*	middle vs. older *p*
Age (years)	26.5 (25.3, 27.7)	44.9 (43.4, 46.4)	71.7 (69.2, 74.1)	-	-	-
BMI (kg/m^2)	24.2 (21.9, 26.5)	26.9 (25.3, 28.6)	26.0 (24.6, 27.4)	0.658	0.390	0.886
Weight (kg)	66.0 (59.6, 72.4)	71.4 (66.4, 76.3)	65.2 (61.2, 69.2)	-	-	-
Height (m)	1.65 (1.62, 1.68)	1.63 (1.60, 1.65)	1.58 (1.56, 1.60)	-	-	-
Body fat (%)	32.5 (28.2, 36.7)	36.3 (33.1, 39.4)	37.9 (34.2, 41.6)	0.977	0.129	0.189
Subcutaneous fat (cm^2)‡	174 (112, 235)	313 (263, 364)	187 (151, 222)	0.619	0.553	0.993
Visceral fat (cm^2)‡	54 (35, 74)	133 (107, 158)	113 (87, 138)	0.259	<0.001	0.032
Steps/day	11588 (9972, 13203)	9836 (7642, 12030)	10547 (8182, 12912)	0.891	0.769	0.967
VO_{2peak} (ml/kg/min)	29.4 (26.6, 32.1)	27.6 (24.6, 30.7)	21.2 (19.2, 23.2)	0.65	<0.001	0.004
VO_{2peak} (ml/kgFFM/min)	43.3 (41.0, 45.5)	43.2 (39.8, 46.5)	34.2 (31.6, 36.8)	0.351	<0.001	0.008
WR_{max} (W/min)	148 (140, 157)	155 (141, 169)	98 (89, 106)	0.840	<0.001	<0.001
Resting RER	0.94 (0.90, 0.97)	0.98 (0.94, 1.01)	0.92 (0.88, 0.96)	0.555	0.837	0.897
Fasting glucose (mmol/L)	5.1 (4.9, 5.3)	4.4 (4.2, 4.6)	5.2 (5.0, 5.3)	0.001	0.903	<0.001
Fasting insulin (µIU/mL)	8.2 (6.5, 9.8)	6.2 (4.4, 8.0)	5.8 (4.3, 7.3)	0.052	0.107	0.912
HOMA-IR	1.90 (1.47, 2.31)	1.21 (0.84, 1.58)	1.36 (1.00, 1.73)	0.015	0.136	0.577
2-hour glucose (mmol/L)	4.7 (4.2, 5.2)	4.7 (4.4, 5.1)	6.1 (5.5, 6.8)	1.000	0.001	<0.001
IHTAG (%)‡	1.86 (1.44, 2.28)	2.04 (1.44, 2.63)	3.88 (2.36, 5.39)	0.862	0.02	0.067

Results shown as means (95% confidence interval). To avoid unnecessary statistical comparisons, height, weight, and age were not compared.
‡ Means and confidence intervals are provided as original data, but statistical comparisons were performed on natural log transformed data. **Abbreviations:** IHTAG, intrahepatic triacylglycerol concentration.

Table 4.2: Volunteer Comparison by Age and Activity Category

	20-30 Years		40-50 Years		≥65 Years	
	Sedentary n of 10	**Active n of 11**	**Sedentary n of 10**	**Active n of 11**	**Sedentary n of 9**	**Active n of 10**
Age (years)	25.0 (23.0, 26.9)[c,d,e,f]	27.9 (27.1, 28,8)[c,d,e,f]	45.6 (43.1, 48.1)[a,b,e,f]	44.3 (42.6, 46.0)[a,b,e,f]	74.1 (70.8, 77.4)[a,b,c,d]	69.5 (66.4, 72.6)[a,b,c,d]
BMI (kg/m^2)	27.4 (23.8, 31.1)[b]	21.3 (19.7, 23.0)[a,e]	26.2, (23.8, 28.6)	24.7 (22.5, 26.9)	27.8 (26.4, 29.3)[b]	24.4 (22.5, 26.2)
Weight (kg)	73.0 (61.7, 84.3)	59.7 (55.3, 64.1)	72.07 (66.2, 78.0)	68.8 (60.7, 76.8)	70.6 (66.0, 75.1)	60.3 (55.5, 65.1)
Height (m)	1.63 (1.58, 1.67)	1.67 (1.64, 1.71)	1.66 (1.63, 1.70)	1.67 (1.64, 1.70)	1.59 (1.57, 1.61)	1.57 (1.54, 1.61)
Body fat (%)	40.8 (35.9, 45.8)[b,d]	25.0 (22.5, 27.5)[a,c,e,]	35.8 (31.8, 39.8)[b]	30.6 (26.1, 35.1)[a,e]	42.7 (40.0, 45.3)[b,d,]	33.7 (28.3, 39.1)
Subcutaneous fat (cm^2)‡	263 (160, 366)[b]	93 (71, 114)[a,c,e]	238 (164, 311)[b]	153 (92, 214)	224 (175, 274)[b]	151 (107, 194)
Visceral fat (cm^2)‡	83 (51, 115)[b]	28, (19, 37)[a,c,e,f]	93 (56, 129)[b]	60 (25, 94)[e]	141 (101, 182)[b,d]	90 (70, 109)[b]
Step Count	8597 (7710, 9484)[b,d,f]	14306 (12474, 16139)[a,c,e]	6100 (5515, 6685)[b,e]	15294 (13671, 16917)[a,c,e]	6219 (4629, 7808)[b,d,f]	14442 (12084, 16801)[b,d,e]
VO_{2peak} (ml/kg/min)	23.6 (21.9, 25.4)[b,d,e]	34.6 (32.4, 36.7)[a,c,e,f]	22.2 (20.7, 23.7)[b,d,]	32.7 (29.0, 36.4)[a,c,e,f]	18.0 (16.6, 19.4)[a,b,d,f]	24.1 (21.8, 26.5)[b,d,e]
VO_{2peak} (ml/kgFFM/min)	40.2 (37.9, 42.5)[e]	46.0 (43.0, 49.1)[c,e,f]	34.9 (32.2, 37.6)[b,d,]	45.6 (41.7, 49.5)[c,e,f]	31.4 (29.2, 33.6)[a,b,d,]	36.7 (32.8, 40.6)[b,d]
WR_{max} (W/min)	135 (126, 144)[b,d,e,f]	160 (151, 170)[a,c,e,f]	127 (120, 134)[b,d,e]	176 (160, 192)[a,c,e,f]	86 (80, 92)[a,b,c,d]	108 (96, 121)[a,b,d]
Resting RER	0.93 (0.89, 0.97)	0.94 (0.87, 1.00)	0.91 (0.86, 0.96)	0.90 (0.86, 0.94)	0.94 (0.87, 1.01)	0.90 (0.86, 0.95)
Fasting glucose (mmol/L)	5.1 (4.9, 5.3)	5.1 (4.8, 5.5)	4.6 (4.3, 4.9)	4.6 (4.3, 4.9)[e,f]	5.2 (4.9, 5.4)[d]	5.2 (4.9, 5.4)[d]
Fasting insulin (µIU/mL)	8.5 (6.2, 10.9)	7.8 (5.5, 10.1)	5.8 (3.3, 8.2)	4.9 (2.2, 7.6)	6.7 (4.3, 9.0)	5.1 (3.2, 6.9)
HOMA-IR	1.99 (1.37, 2.60)	1.80 (1.22, 2.39)	1.15 (0.66, 1.63)	0.99 (0.41, 1.57)	1.55 (0.98, 2.11)	1.19 (0.71, 1.67)
2-hour glucose (mmol/L)	5.1 (4.5, 5.8)	4.3 (3.7, 4.9)[e,f]	4.9 (4.4, 5.4)	4.5 (3.9, 5.0)[e]	6.5 (5.4, 7.5)[b,d]	5.9 (5.0, 6.7)[b]
IHTAG (%)‡	2.1 (1.7, 2.4)	1.7 (0.9, 2.4)[e]	2.2 (1.5, 2.9)	2.2 (1.2, 3.1)	4.7 (2.1, 7.2)[b]	3.2 (1.4, 4.9)

Results shown as means (95% confidence interval). For basic anthropometry only BMI was compared across groups. **Abbreviations:** BMI, body mass index; FFM, fat free mass; HOMA-IR, homeostasis model of insulin resistance; hr, hour; min, minute; RER, respiratory exchange ratio; SBP, systolic blood pressure; VO_2, volume of oxygen; W, watts; WR, work rate;

‡ Means and confidence intervals are provided as original data, but statistical comparisons were performed on natural log transformed data.
[a] significantly different from sedentary 20-30 year olds ($p<0.05$).
[b] significantly different from active 20-30 year olds ($p<0.05$).
[c] significantly different from sedentary 40-50 year olds ($p<0.05$).
[d] significantly different from active 40-50 year olds ($p<0.05$).
[e] significantly different from sedentary ≥65 year olds ($p<0.05$).
[f] significantly different from active ≥65 year olds ($p<0.05$).

Table 4.3: Correlations of Age, Physical Activity and Fitness, Body Composition and Fat Distribution, and Glucose Control

	VO_{2peak} by FFM	Body fat %	Visceral fat	IHTAG	Fasting glucose	Fasting insulin	2-hour glucose
Age	-0.519**	0.274*	0.409**	0.246	0.082	-0.244	0.393**
Step count	0.513**	-0.564**	-0.447**	-0.263*	-0.023	-0.082	-0.268*
VO_{2peak} by FFM		-0.319*	-0.452**	-0.346**	-0.033	0.098	-0.177
Body fat %			0.627**	0.321*	0.178	0.114	0.527**
Visceral fat area				0.546**	0.182	0.238	0.483**
Intrahepatic lipid					0.251	0.372**	0.248
Fasting glucose						0.330*	0.245
Fasting insulin							0.039

Spearman's Rank correlations of unadjusted variables. **Abbreviations:** FFM, fat free mass; IHTAG, intrahepatic triacylglycerol concentration.
* $p \leq 0.05$
** $p \leq 0.01$

4.3.2 Body Composition and Liver Fat by Age and Physical Activity Category

Results by age and physical activity category are shown in *Table 4.2.* Young active women had significantly lower body fat than sedentary women of any age category. There was a trend toward a difference between young active women and older active women ($p = 0.052$). Active middle-aged women had lower body fat than sedentary young and sedentary older women, but they were not significantly different from their sedentary middle-aged counterparts.

Active young women had significantly less subcutaneous abdominal fat than sedentary women in all age categories. However, active middle-aged and older women did not differ significantly from their sedentary counterparts. Young active women had significantly less visceral fat than all other groups except active middle-aged women. Active middle-aged women had significantly less visceral fat than sedentary older women. Visceral fat area was similar across all sedentary groups. Active young women also had lower IHTAG than sedentary older women, but this difference was no longer significant after adjustment for either $VO_{2peakFFM}$ or body fat percentage. No other differences between groups reached statistical significance before or after adjustment. Step count was inversely correlated with body fat percentage ($r = -0.564$, $p \leq 0.01$), visceral fat ($r = -0.447$, $p \leq 0.01$), IHTAG ($r = -0.263$, $p \leq 0.05$)(*Table 4.3*). Whereas, $VO_{2peakFFM}$ was inversely correlated with body fat ($r = -0.319$, $p \leq 0.05$) and visceral fat ($r = -0.452$, $p \leq 0.01$) (*Table 4.3*).

4.3.3 Metabolism, Glucose Control, and Insulin Sensitivity by Age

Results by age category are shown in Table *4.1.* Resting respiratory exchange ratio and fasting insulin were similar across age categories. Fasting glucose was lowest in middle-aged women and similar between young and older women. HOMA-IR was higher in young than in middle-aged women. Two-hour glucose concentration was highest in older women, but similar in young and middle-aged women. Age was associated with 2-hour glucose, but not fasting glucose or insulin (*Table 4.3*).

4.3.4 Metabolism, Glucose Control, and Insulin Sensitivity by Age and Physical Activity Category

Results by age and physical activity category are shown in *Table 4.2.* Resting respiratory exchange ratio was similar across all groups. Two-hour glucose was lowest in active young women and highest in sedentary older women with significant differences between active young and active middle-aged women relative to sedentary older women. Fasting glucose was lower in active middle-aged women than older women, but similar across all other groups. Fasting insulin and HOMA-IR were similar across all groups. Neither adjustment for $VO_{2peakFFM}$ nor body fat percentage altered these findings. Step count was inversely correlated with 2-hour glucose concentration (r = -0.447, $p \leq 0.01$), but not fasting glucose or insulin. However, $VO_{2peakFFM}$ was not correlated with fasting or 2-hour glucose, or fasting insulin.

4.3.5 Cardiorespiratory Fitness and Work Capacity by Age

Results by age category are shown in Table *4.1.* Cardiorespiratory fitness as assessed by VO_{2peak} relative to both total body weight and fat free mass, and work capacity assessed by WR_{max} were similar between young and middle-aged women but lower in older women by approximately 25%, 21%, and 35%, respectively. Age was inversely correlated with $VO_{2peakFFM}$ (r = -0.519, $p \leq 0.01$).

4.3.6 Cardiorespiratory Fitness and Work Capacity by Age and Physical Activity Category

Results by age and physical activity category are shown in *Table 4.2.* Cardiorespiratory fitness expressed as VO_{2peak} was 47% higher in active young and middle aged women, and 34% higher in active older women relative to their sedentary peers. Adjusting for body composition by using $VO_{2peakFFM}$ substantially reduced this difference especially in young and older women: $VO_{2peakFFM}$ was 14%, 31%, and 18% higher in active young, middle-aged, and older women, respectively. WR_{max} was 19%, 39%, and 26% higher in active young, middle-aged, and older women, respectively. However, the difference

between active and sedentary older women did not reach significance (p = 0.094).

Sedentary young and middle-aged women had similar $VO_{2peak,}$ $VO_{2peakFFM}$, and WR_{max}, but these parameters were all higher than in sedentary older women, and WR_{max} was also higher than in active older women. Active young and middle-aged women had comparable $VO_{2peak,}$ $VO_{2peakFFM}$, and WR_{max}, but both groups had higher values than sedentary and active older. Step count correlated with $VO_{2peakFFM}$ (r = 0.513, $p \leq 0.01$)

4.3.7 Correlations Between Body Composition, Fat Distribution, and Glucose Control

All correlations are shown in *Table 4.3*. Body fat was strongly correlated with visceral fat (r = 0.627, $p \leq 0.01$) and 2-hour glucose (r = 0.527, $p \leq 0.01$), and more modestly with IHTAG (r = 0.321, $p \leq 0.05$), but not fasting glucose or insulin. Visceral fat correlated with IHTAG (r = 0.546, $p \leq 0.01$) and 2-hour glucose (r = 0.483, $p \leq 0.01$), but not fasting glucose or insulin. IHTAG correlated with fasting insulin (r = 0.372, $p \leq 0.01$), and almost significantly with fasting and 2-hour glucose (r = 0.25, p = 0.051 and r = 0.248, p = 0.054, respectively).

4.4 Discussion

This cross-sectional study presents physical fitness and regional fat data from young, middle-aged and older women subdivided into sedentary and physically active groups. The data suggest a decline in physical fitness from the middle-age onwards. Physical activity did not attenuate the decline in cardiorespiratory fitness but was associated with a higher starting point. Cardiorespiratory fitness was inversely associated with adiposity even when calculated relative to fat free mass. Liver fat showed a tendency to increase with age.

The most consistent finding across groups was a positive association between physical activity and fitness. However, older women had markedly lower cardiorespiratory fitness than their younger counterparts even when physical activity was comparable. Overall, age was inversely associated with cardiorespiratory fitness. Comparison of groups sub-divided by age and physical activity indicated that being active somewhat attenuated the decline in fitness among older women but did not eliminate it entirely.

Other cross-sectional work suggests VO_{2peak} declines by 8-10% per decade in healthy men and women [546,547]. Longitudinal observation confirms this decline [548], and shows it to be non-linear as it accelerates to a 20-25% reduction in VO_{2peak} per decade in those over 70 years [549,550]. As these findings were in apparently healthy populations, they suggest an inevitability of this decline. Indeed, although an active lifestyle including regular exercise is associated with a higher cardiorespiratory fitness across the lifespan [551], a decline with age is still evident in both men [552], and women [553], and may actually be greater in absolute terms among regular exercisers.

Nonetheless, the ability to respond to aerobic exercise is maintained in populations over 60 years with a meta-analysis reporting an average improvement in VO_{2peak} of 16% [554]. Some attenuation of response with age has been demonstrated in postmenopausal women [555]. However, increasing exercise volume increased the likelihood of a clear improvement in cardiorespiratory fitness in that cohort [556]. As no data on the menstrual status

was collected, and women regularly engaging in planned exercise were excluded, the present dataset cannot address these issues.

4.4.1 Age, Physical Activity and Body Composition

In the present cohort, physical activity was inversely associated with total as well as regional fat, and age was weakly positively associated with body fat percentage. The correlation was stronger for visceral fat and age suggesting a shift in fat distribution from areas less to areas more closely linked with all cause mortality and cardiometabolic risk [557]. Prior cross-sectional and longitudinal observations have shown similar inverse associations between physical activity and body fat [558-563], and several longitudinal studies have shown an increase in fat mass and decrease in lean mass with age [564]. Others have suggested that women have an annual increase in fat mass from 40-66 years of approximately 0.41 kg/year [561], or a 7.5% increase per decade in those over 45 years [565]. One of the mediators of this change in body composition is menopause, which was more closely associated with increasing fat mass than age [561]. As in our cohort, others have reported an age related shift in fat distribution to one more dominated by central, and specifically visceral abdominal fat [566]. Despite a tendency for a gradual increase in adiposity over several decades, there appears to be a decline after the age of 80 years [567]. No data on menstrual status or history were collected in the present cohort, and the age range in the ‘older’ group did not include a sample set of women over 80 years.

The decline in fat free mass has previously been shown to become more marked after the age of 45 years [568]. Between 40-66 years this is approximately 0.06-0.07 kg/year in healthy adults [561,569], but followed by an accelerated decline in the seventh decade of life [570]. After the seventh decade of life there is clear trend toward total body weight reduction predominantly from fat free mass with increasing infiltration of muscle with adipose tissue, i.e. myosteatosis [571]. This phenomenon, coined as *sarcopenic obesity*, is not only associated with HOMA-IR [572], but leads to substantial deficits in functional capacity and risk of mortality [573].

The present cohort may have been too young, on average, to detect such a major shift in body composition beyond the weak correlation. The moderating influence for physical activity in this age-related tendency to increase fat mass identified in Guatemalan women aged 20-87, a population in whom relatively high levels of physical activity are maintained throughout life [574], also suggests that activity was a attenuating factor in the present cohort. However, the present study and that done in Guatemalan women used different and not readily comparable measures of physical activity, and it seems probable that women in Guatemala were more physically active in 1997 than women in United Kingdom in 2010. Notably, higher physical activity did not attenuate the decline in fat free mass in Guatemalan women [574].

The most plausible explanation for us not observing a greater shift in body fat percentage or stronger correlations is the secular trend in body fatness. Given the population wide trajectory of obesity has been upward for some decades and there appears to be a generational lag [567], it seems plausible that any natural trend in age-related changes to body composition are interacting with non-age related factors [575].

Collectively our observations and those of others highlight the importance of physical activity throughout the adult lifecycle in terms of attenuating the increase in fat mass and the associated health risks over time. However, further work is warranted to see what approach to physical activity is both effective and feasible for large portions of our ageing population.

4.4.2 Insulin Sensitivity and Age

Fasting glucose and insulin were mostly too insensitive a measure to detect differences in insulin sensitivity and glucose control between groups in the present cohort. This was consistent with the deliberate exclusion of volunteers with metabolic disease. Nonetheless, the pooled aged group comparison showed young women to have the highest degree of fasting insulin and HOMA-IR with similar fasting glucose to older women, but a normal blood glucose response to the oral glucose tolerance test. Interestingly, dividing young women into sedentary and active groups revealed no significant intergroup differences

in any of the measures of glucose control. The presence of insulin resistance in young women was likely compensated by increased insulin production to maintain glucose control, whereas in older women any capacity to compensate for insulin resistance was reduced. Middle-aged women had the HOMA-IR and comparable glucose control to young women. Older women had the highest 2-hour glucose response, and age showed a modest significant correlation with 2-hour glucose indicating poorer glucose control with age.

The observed trend in reduced glucose control with age in the present cohort is reflected by the increase in type 2 diabetes with age observed by others [576], and similar observations in larger cross-sectional studies also excluding volunteers with diabetes. In 20 European cohorts encompassing men and women aged 18-85 years with normal glucose tolerance insulin action, as assessed by the reference standard hyperinsulinaemic-euglycaemic clamp, declined with age, but adjusting for BMI removed this relationship [577]. However, in the absence of more direct assessment of body fat and its distribution, a BMI ≤ 25 kg/m^2 was used to define the ‘lean’ group which in turn was used to derive the researchers definition of insulin resistance. At these lower BMIs, the correlation between BMI and body composition is poor [578,579], which puts the chosen definition in question. Other reports support the findings in the present study by indicating that age, independent of gender, insulin sensitivity, fasting glucose, BMI and waist-to-hip ratio, is associated with a progressive decline in both basal insulin release and systemic insulin clearance [580].

A pooled analysis of 10 non-diabetic European cohorts aged 30-88 years and adjusted for BMI showed an increase in HOMA-IR assessed insulin resistance as well impaired fasting glucose and impaired glucose tolerance with age; the latter being the strongest association [581]. Age was also inversely associated with insulin sensitivity assessed by HOMA-IR independent of BMI and waist hip ratio in mexican women aged 30-65 years [582]. A French cohort of men and women aged 30-64 years has also showed an increased fasting insulin with age; division of the cohort into four age classes indicated that the rise occurred after the age of 49 years in women and 59 years in men [583]. And a longitudinal evaluation in US men and women aged over 30 years with normal glucose tolerance at baseline and followed up for 4-11 years suggested that baseline

insulin resistance, assessed by insulin suppression test, is predictive of age related disease incidence [584].

The frequently observed decrease in insulin activity with age has been attributed to the aforementioned changes in body composition and fat distribution, and declining physical activity [585]. Ectopic fat in the liver (see *Section 4.4.5*) and the pancreas have emerged as likely mediators of central insulin resistance, and impaired insulin response, respectively [586]. Interestingly, HOMA-IR in a healthy cohort of Italian men and women aged 28-110 years, was highest in those aged 80-90 years followed by those aged 50-60, while centenarians had the lowest average of any group closely followed by nonagenarians [587]. However, in the same cohort HOMA-β indicated a rise in pancreatic β-cell function across age groups with a peak in octogenarians and a marked decline in nonagenarians and centenarians. Further, evaluation of the Framingham and Framingham offspring cohorts showed those participants living for 90 or more years had lower blood glucose, form either spot or fasting samples, were lower for all time points after approximately 50 years than for those dying earlier [588]. Thus although an age-related decline in insulin sensitivity and glucose control is seen across much of the age spectrum, observations of the most long-lived suggest this decline need not always be rapid and lead to morbidity or mortality.

The elevation of 2-hour glucose with age, observed in the present and other cohorts is noteworthy, as 2-hour glucose when exceeding fasting glucose, even within the normoglycemic range, is associated with increased cardiovascular disease mortality [589,590].

4.4.3 Insulin Sensitivity and Physical Activity

In the present cohort, comparisons between sedentary and active women showed no significant effect of physical activity. However, there was an inverse correlation between step count and 2-hour glucose. The latter finding is more consistent with expectations as the ability of exercise to improve insulin sensitivity and glucose control across a range of ages is well established (see *Section 1.10*). In brief, physical activity, particularly moderate to vigorous

aerobic activity, has been demonstrated to increase both insulin mediated and insulin independent glucose uptake by muscle, the latter only being observed for approximately 60 minutes post exercise [591]. The insulin sensitising is localised to contracting muscle [591], and has been shown to last 48 hours but fewer than five days [592]. Even in well-trained endurance athletes displaying twice the whole-body glucose uptake rate of sedentary controls 12 hours post-exercise, cessation of training for just three days abolished substantially reduced this difference and 7 days of detraining abolished it [593]. Further, nutrient intake is a major mediator of this effect as 100 g oral glucose [508], but not fat [509,510], within three hours of exercise has been shown to prevent the next-day increase in insulin-mediated glucose disposal. Both the localised effect and its attenuation by restoration of muscle glycogen with post-exercise glucose administration indicate glycogen depletion is an important component of myocellular insulin sensitisation. Thus the difference in amount or intensity of physical activity in the present cohort may not have been sufficient to notice a clear difference, especially given glucose control throughout the cohort was mostly within the normal range.

Non-exercise physical activity, such as that assessed in the present study, has been previously reported to be associated with insulin and glucose metabolism. Early work established an association between habitual physical activity, assessed by 7-day recall, and fasting insulin in non-diabetic hispanic and non-hispanic caucasian US men aged 20-75 years, but not in women [594]. Similarly, in a Canadian cohort of men and women aged 18-79 years, physical activity assessed by past-year recall was associated with fasting insulin, independent of age, BMI, body fat percentage and waist circumference, but only in men [595]. The authors of both studies suggested this gender discrepancy may have arisen from either the small distribution of physical activity in the female cohort, or poorer validity of the questionnaire for women [594,595]. Other studies have not reported this degree of gender discrepancy.

In a US cohort of men and women aged 40-69 years and including volunteers with normal glucose tolerance, impaired glucose tolerance, and non-insulin dependent type 2 diabetes, assessed by physical activity recall, vigorous, non-vigorous, and total physical activity were associated with insulin sensitivity

measured by intravenous glucose tolerance [596]. Questionnaire assessed physical activity was also associated with mean insulin – average of fasting and 2-hour post glucose challenge – in nondiabetic samples from two populations with differing body compositions, Pima Indians and Mauritians [595].

All-type physical activity was also inversely associated with insulin sensitivity across the lifecycle [563,597,598]. Both category, leisure time and work-related, and intensity of physical activity mediate this relationship. In a Swedish sample, leisure time physical activity was inversely associated with insulin sensitivity independent of age and the association was stronger in women than in men [563]. In Iranian women, after adjustment for age and BMI, total physical activity was most closely associated with insulin sensitivity followed by an inverse association with sedentary time, and positive association with the duration of high-intensity activity after adjusting for confounders including age, gender, waist circumference, and smoking [597]. The association with light physical activity remained after adjustment for moderate-to-vigorous activity.

NHANES data from 2003-2006 showed an association and inverse association with fasting insulin for questionnaire assessed time spent watching television and leisure time physical activity, respectively, but no such association with transportation or household physical activity [599]. Conversely, self-reported physical activity in NHANES 1999-2002 was not associated with insulin sensitivity [600], but this may be partly explained by the presence of non-fasting samples in the analysis as fasting status was not clearly described.

A similar relationship was shown in a large Australian cohort of non-diabetic men and women over 35 years in whom television viewing time was associated with 2-hour glucose, fasting insulin, HOMA-IR and HOMA-β, but only in women [601]. Seven-day accelerometry in a small subset of this Australian cohort showed sedentary time to be associated with 2-hour glucose, with an inverse association for light and moderate-to-vigorous intensity independent of age, gender, height, waist circumference [602]. The positive effect of light activity remained even after adjusting for moderate-to-vigorous intensity.

In middle-aged US women 7-day accelerometry assessed time spent doing moderate-intensity and vigorous-intensity activity was inversely related to and sedentary time related to HOMA-IR [603]. Adjusting for air displacement plethysmography assessed body fat percentage or waist circumference removed these associations. In a multi-country cross-sectional European cohort assessing physical activity by accelerometry total activity was associated with insulin sensitivity measured by 2-hour hyperinsulinemic-euglycemic clamp [604]. Sedentary time, intensity of activity, and time spent doing light activity were also associated with insulin sensitivity, but this association disappeared after each variable was adjusted for total activity.

In a longitudinal observation of healthy middle-aged men and women in the UK, sedentary time, assessed by heart rate measurement, at baseline predicted fasting insulin at followup; independent of age, gender, fat mass, fasting insulin, and smoking status at baseline [605]. However in cross-sectional assessment of another UK cohort of non-diabetic men and women aged 50-75 years, time spent moderately to vigorously physically active defined as 1.75 and 2 times resting heart rate, respectively, was inversely associated with fasting insulin whereas time spent doing light activity, defined as 1.5 times resting heart rate, was not [606]. Interestingly, in UK men aged 42-64 although only overall body fat was associated with whole body insulin sensitivity after taking into account physical activity energy expenditure and VO_{2peak}, but only physical activity energy expenditure was associated specifically with hepatic insulin sensitivity [607].

The importance of sedentary time was recently confirmed by work showing that breaking up sedentary time with 2-minute bouts of light- or moderate-intensity walking reduced postprandial glucose and insulin response in overweight and obese 45-65 year old men and women [25].

Discrepancies in the associations reported may be due to differences in the actual cohorts, but are also to methodological differences. Older studies relied more on physical activity recall. Larger cohorts similarly often rely on recall, and also simple biomarker measures such as fasting blood glucose and insulin measurements, which are less sensitive and less specific than the

hyperinsulinaemic-euglycaemic clamp or oral glucose tolerance test [512]. Even with the employment of accelerometers or heart rate monitors to provide more objective assessments of physical activity, there were differences in devices used and the parameters employed to define categories of physical activity.

Despite these variations in methodology and some inconsistency in findings, overall physical activity of all intensities and types appears to improve insulin sensitivity and glucose control. Conversely, time spent sedentary, especially if uninterrupted for prolonged periods, reduces insulin sensitivity and glucose control. These associations are somewhat attenuated when controlling for body composition, but they are not eliminated. Interestingly, a recent meta-analysis reported that television viewing time was causally associated with excessive food intake [608]. Such a finding highlights that physical activity does not exist in a vacuum, and changing it is likely to lead to other lifestyle changes that may be unanticipated, but potentially quite beneficial.

4.4.4 Insulin Sensitivity and Body Composition

In the present cohort, body fat percentage and visceral fat area were closely associated with 2-hour glucose, and IHTAG with fasting insulin; suggesting some independence of peripheral and central insulin resistance. The inverse association between obesity, particularly central obesity, and insulin sensitivity is a frequent observation[609-612]. Even in the absence of overt insulin resistance, findings from the EGIR pooled cohort showed increased insulin hypersecretion among obese volunteers, especially centrally obese women [613]. However, the inverse association of total body fat and insulin sensitivity is not universal as there are those displaying normal glucose and insulin metabolism despite being obese. Such obese but *metabolically healthy* individuals have less central adiposity than those with insulin resistance and/or dyslipidaemia [614-617].

When measured, the association between visceral fat and whole-body insulin sensitivity is particularly strong [618-620], but the association between visceral and IHTAG is also very strong [238,621,622]. Recent work assessing the relationship of visceral fat, IHTAG, and insulin sensitivity/glucose control, but with conflicting findings. One report suggests visceral fat is independently associated with

glucose control and liver fat is not [238]. Two reports in much larger cohorts suggests that visceral fat and IHTAG are independently associated with insulin sensitivity and/or glucose control [622,623]. Further, IHTAG is inversely associated with hepatic insulin sensitivity [120,624-626].

4.4.5 Liver fat

In the present cohort IHTAG was higher in older women than young women. However, only seven women had an IHTAG ≥5%: five in the older group (3 sedentary and 2 active); one in the young (active) group. Subdividing the cohort by physical activity in addition to age maintained this age difference in liver fat but only in active young women relative to sedentary older women. Consistent with this observation, IHTAG was inversely associated with $VO_{2peakFFM}$ and step count, but not age. This suggests physical activity and or cardiorespiratory fitness are modifiers of liver fat rather than chronological age.

The prevalence and severity of fatty liver has previously been shown to increase with age [183,187,201]. This relationship has been shown in a range of populations in: Northeast England [201]; Hong Kong [187], and Texas [183]; India [627]; China [157]; and Korea [191]. However, none of these surveys has controlled for physical activity or cardiorespiratory fitness.

Italian cross-sectional data have shown physical activity to also be associated with the prevalence of NAFLD even after adjustment for age, gender, BMI, and insulin sensitivity [232]. Cross-sectional data from Israel confirmed this association, however after adjustment for BMI, only the association with resistance training remained [233]. Longitudinal data, also from Israel, showed that total leisure time physical activity and anaerobic/resistance training physical activity to be lower in those going on to develop NAFLD [237]. Further, retrospective analysis of a cohort with NAFLD showed those meeting US guidelines for vigorous exercise to be less likely to have NASH [240]. However, in this same cohort no such association was shown for moderate-intensity or total exercise. There is also an inverse association between liver fat and cardiorespiratory fitness [238], i.e. VO_{2peak}. Indeed, NAFLD was found to be ~10-times more prevalent in the lowest relative to the highest tertile of

cardiorespiratory fitness independent of BMI, though not independent of waist circumference [239]. Further, cardiorespiratory fitness was identified as an independent predictor of liver fat reduction in lifestyle therapy [8]. Further, exercise only interventions using aerobic exercise or resistance training have both been shown to reduce liver fat independent of weight reduction [243-245] (see *Chapter 2* for a more detailed discussion).

Our observations and those of others highlight the protective effect of physical activity and both cardiorespiratory fitness and muscle fitness – strength – on liver health. These observations also identify the likely problem of confounding when physical activity and fitness are not considered in the establishment of risk factors for NAFLD.

4.4.6 Strengths and Limitations

The cross-sectional nature of the present study precludes inference about the direction of causality in the apparent relationships, while the small sample size limits statistical power generally, and limits subdivision of groups to more closely assess the mediators of the apparent relationships. Also, the older category allowed for a greater heterogeneity of age as it was not limited to a ten year span like the young and middle-aged category were. The absence of dietary intake data precludes identification of this plausible mediator of the findings. The use of a single-centre self-selected sample excluding those with clinical symptoms and those participating in regular exercise physical activity, limits the extrapolation of the results to the wider community, which encompasses a much broader range of lifestyles than this study is able to reflect. The device used to assess step count has not been formally validated for this purpose. Further, recent work suggests that distance walked is an important determinant of adiposity yet step count does not provide information on actual distance covered [628]. Pooling age groups despite each containing two intentionally distinct groups in terms of physical activity may also confound comparisons between age groups. Despite these limitations the relatively strong correlations consistent with findings in larger cohorts suggests that the study was sufficiently large and step counts adequate to identify close relationships.

Conversely, applying the specific criteria of age, sex, amount of physical activity, and health status ensured a degree of homogeneity within and between groups, which strengthens the ability to extrapolate results to similar groups in the community. Also, in contrast to longitudinal research a cross-sectional approach is not prone to confounding by environmental changes over the time, e.g. increasing prevalence of obesity [567], and increasing fasting glucose independent of age [576].

Use of an objective measure physical activity and use of high quality quantitative methods to assess, cardiorespiratory fitness, body composition and fat distribution, and liver fat improves the methodological rigour of this study relative to larger cohort studies that have tended to use self-reported physical activity, no or more basic measures of cardiorespiratory fitness, and less accurate measures of body composition, fat distribution, and IHTAG. A further strength is that, in addition to controlling for cardiorespiratory fitness statistically, women taking part in regular planned exercise were excluded so that the established relationships are with non-exercise physical activity.

4.5 Conclusion

Ageing in the present and other cohorts, even in the absence of disease, is consistently accompanied by reductions in cardiorespiratory fitness, muscle strength and mass, and functional capacity. Ageing is also frequently accompanied by increased fat mass, increased hepatic steatosis, decreased insulin sensitivity, and reduced glucose control. These changes are closely associated with morbidity and mortality irrespective of age. Fortunately, they are all at least partly amenable to improvement via appropriate physical activity, both general and targeted exercise, as well as reductions in uninterrupted sedentary time. Public health focus should therefore be on increasing physical activity, cardiorespiratory fitness, and muscle strength, and reducing total and uninterrupted sedentary time in the whole population throughout the lifecycle.

Chapter 5. High-Intensity Intermittent Training in Non-Alcoholic Fatty Liver Disease

5.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of liver disease in much of the World with an estimated prevalence in Western countries of 20-30% in the general adult population, and greater prevalence among certain ethnic groups, the obese, and those with type 2 diabetes [5,629] (see *Section 1.6* & *1.7*). A recent UK audit of 1118 primary-care patients indicated a prevalence of 26.4% [630]. However, this is likely to be an underestimate as steatosis was assessed by ultrasound, a relatively insensitive technique (see *Section 1.4.4*), and assessments were done based on abnormal liver enzymes which are also insensitive to the presence of hepatic steatosis (see *Section 1.4.5*).

NALFD is closely associated with the symptoms of the metabolic syndrome, notably central obesity, impaired glucose control, elevated blood pressure, hypertriacylglyerolaemia, and low high-density lipoprotein [2,270]. Thus the condition can progress from simple steatosis to non-alcoholic steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma, but is also closely linked to the development of type 2 diabetes and cardiovascular disease [629](see *Section 1.8*).

The recommended, and in the absence of approved pharmacotherapy only, treatment for NAFLD, is lifestyle intervention with an emphasis on weight reduction [14,311]. However, although numerous dietary approaches exist that are effective at achieving weight reduction (see *Sections 2.3.2.1* and *2.4*), weight maintenance proves challenging for the majority of people [631]. This is true even in well funded multifactorial lifestyle interventions [358]. Weight reduction *per se* may also not be called for in patients with NAFLD but normal body weight. The importance of physical activity and physical fitness for the prevention and treatment of NAFLD has become apparent with cross-sectional and prospective cohort analyses reporting an inverse association of physical activity and cardiorespiratory fitness with risk of NAFLD [232,233,237-239]. Further, retrospective

analysis of a cohort with NAFLD showed those meeting US guidelines for vigorous exercise were less likely to have NASH [240]. However, the capacity to perform vigorous activity in patients with NASH is impaired consistent with disease severity [181].

Recent small scale exercise only interventions have demonstrated the potential for exercise to modestly reduce liver fat independently of weight change and dietary modification [243-245] (see Chapter 2 for extensive discussion). The capacity for exercise to improve glucose control is well established [274,408]. However, exercise interventions in patients with objectively and quantitatively assessed liver fat are few and have tended to conduct partly or fully supervised sessions that are too resource intensive to be translated into clinical care [244,245]. No study has previously assessed high- intensity intermittent training (HIIT) in a cohort with clinically defined NAFLD. HIIT is time efficient, has a good safety record (see *Section 3.3.1*), and is highly effective at improving fitness in a broad range of healthy and clinical populations (see *Section 3.3.2*). HIIT has also been shown, albeit less consistently, to reduce blood pressure (see *Section 3.3.2.3* & *3.3.2.3*), improve glucose control (see *Section 3.3.4.1* & *3.3.4.2.1*), and reduce body fat (see *Section 3.3.3*).

Given the successful track record of HIIT in improving many of the comorbidities of NALFD, this study aimed to define the effect of 12 weeks of thrice weekly HIIT on adults with NAFLD; specifically with respect to:

1. intrahepatic triacylglycerol concentration (IHTAG)
2. glucose control
3. substrate utilisation during rest and submaximal exercise
4. liver enzyme profile
5. fasting blood lipid profile
6. body composition; and
7. blood pressure

The *null hypothesis* was that there would be no change in liver fat or other measured parameters over time between the HIIT intervention and a control group given standard care.

5.2 Methods

5.2.1 Design and Recruitment

This was a single-centre quasi-randomised controlled trial with a parallel design. The randomisation was broken in one volunteer who was identified as having previously undiagnosed type 2 diabetes and would have been lost to the study if allocated to the control group and given standard care. The study aimed to enrol 28 sedentary adults aged 18-70 years with non-alcoholic fatty liver disease. Excess liver fat was defined as >5% [124], and advanced liver disease was ruled out by including only volunteers with a NAFLD Fibrosis Score of ≤-1.455, corresponding to a negative predictive value of 88-93% for advanced (Kleiner F3/4) fibrosis [167]. The age range was selected based on apparent safety and efficacy of HIIT in this range reported published studies employing similar exercise protocols in clinical populations (see *Section 3.3.1*).

Exclusion criteria were as follows: pregnancy; inability to adhere to the intervention due to physical or mental factors; contraindications to magnetic resonance scanning; viral hepatitis; uncontrolled thyroid condition; haemochromatosis; mean self-reported alcohol intake exceeding 21 units for men or 14 units for women.; suspicion that pharmacotherapy was the cause of the hepatic steatosis; self-reported or activity monitor assessed physical activity exceeding one hour of vigorous activity (≥ 6 metabolic equivalents) per week; or diabetes medication other than metformin. Participants were also required to have been weight stable and on a stable regime of prescriptions medication for the past six months.

An online random number generator (www.randomization.com) was used to establish a treatment allocation sequence. Eligible volunteers were assigned in order following baseline assessments. When one participant withdrew prior to completion, the next available participant took their place in the sequence.

The study was given ethical approval by the Newcastle and Northeast Tyneside Local Research Ethics Committees. All volunteers were provided with a participant information sheet (see *Appendix 5*) before providing written informed

consent (see *Appendix 6*). Volunteers were recruited from the Northeast of England by referral from a secondary care (Freeman Hospital, Newcastle upon Tyne, UK), advertising in local newspapers, and word of mouth. Recruitment was conducted between August 2010 and March 2012.

Post-intervention assessments took place at least 48 hours following the last bout of exercise to avoid observing a last bout effect.

5.2.2 Volunteer Safety and Eligibility Screening

Prior to taking part in the intervention, volunteers were screened to determine their readiness to undertake the exercise required. Full details of procedures and copies of forms can be found in Supplementary Document 1: Risk Definition and Standard Operating Procedures for Exercise Testing (see *Appendix 6*). A questionnaire was used to establish if magnetic resonance scanning was contraindicated due to implanted ferrous material or other reasons (see *Appendix 7*)

5.2.2.1 Medical History and Physical Examination

Medical history was collected, and physical examination performed as described in *Section 4.2.2.*

5.2.2.2 Maximal Progressive Exercise Test

Exercise testing was carried out as described in *Section 4.2.3.*

5.2.3 Magnetic Resonance Spectroscopy and Imaging of Liver and Abdominal Fat

All magnetic resonance imaging and spectroscopy was done using a 3T Philips Achieva scanner (Philips, Netherlands). Intrahepatic lipid triacylglycerol (IHTAG) concentration was measured by localised proton-energy magnetic resonance spectroscopy (^{1}H-MRS) (PRESS, TR/TR 3000 ms/35 ms, 3x3x3 cm voxel, SENSE torso array). Quantification of the spectra (water and CH2 resonances) was performed using jMRUI version 3.0 (Claude Bernard University, Lyon, France) [632,633]. Following manual first and second order phase correction,

spectra were analysed using a non-linear least squares algorithm (AMARES) [634]. IHTAG was expressed as a percentage of liver volume, corrected for proton density of water and lipid.

Abdominal visceral and subcutaneous fat was measured at L4/L5 using a three-point Dixon sequence (TR/TE/number of averages/flip angle 50 ms/ 3.45, 4.60, 5.75 ms/1/308, matrix 160x109, median field of view (FOV) 440 mm, range 400x480 mm to suit subject size with 70% phase FOV). The slice was acquired during a breath-hold and with slice thickness of 10 mm [535,536]. Fat and water were separated, and binary gating applied to produce a map of structures containing more than 50% fat. ImageJ (National Institute of Health, USA) was used to calculate total, subcutaneous, and visceral fat area by creating a binary image and using a watershed algorithm [537].

5.2.4 Fasted Blood Sample Collection, Storage, and Analysis

All blood samples were collected from antecubital veins by cannulation (22G Vasofix® Cero, B Braun Melsungen AG, Melsungen, Germany). Cannula patency was maintained using mandrins (B Braun Melsungen AG, Melsungen, Germany). Fasted (≥ 10 hours with no food or beverages other than plain water) blood samples were collected into BD K3E and SST™ Advance vacutainers (BD Diagnostics, Plymouth, UK) for analysis of total (TC) and high-density lipoprotein (HDL) cholesterol, triacylglycerols (TAG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT); glycated haemoglobin A1c (HbA_{1c}) and serum ferritin.

Total cholesterol [538], HDL-cholesterol [539], ALT [540], and AST [541] were measured using the Roche Modular P and test kits (Roche Diagnostics Ltd, Burgess Hill, UK). LDL-cholesterol was calculated using the the Friedewald equation [542]. GGT was measured using a Roche Modular E170 [543]. HbA1c was measured using a TOSOH HLC-723G7 (Tosoh Corporation, Tokyo, Japan). Serum ferritin was measured by radioimmunoassay [544]. These analyses were carried out by a Clinical Pathology Accredited (CPA) laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry) on the same day as samples were acquired.

5.2.5 Frequently Sampled Oral Glucose Tolerance Test

Fasting and blood collection was as described above. Participants were given a 350 mL Lucozade Original glucose drink (GlaxoSmithKline, Gloucestershire, UK), corresponding to 75 g of glucose, to consume within three minutes. Blood samples were taken at time: 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, and 120 minutes.

Whole blood glucose was collected into fluoride oxalate tubes (Teklab, Sacriston, UK) and measured immediately with a YSI 2300 Stat Plus-D (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Additional whole blood was collected in K-EDTA tubes (Teklab, Sacriston, UK) and uncoated glass tubes (Teklab, Sacriston, UK), and centrifuged at 3000 rpm (2133 RCF) for 10 minutes in a Sigma 6K15 laboratory centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The resulting serum and plasma was stored at -40 ºC (MDF-U5411 plasma freezer, Sanyo Electric Biomedical Co. Ltd., Osaka, Japan) in Nalgene cryogenic vials (Thermo Fisher Scientific, Rosklide, Denmark) until analysis of plasma insulin and serum non-esterified fatty acids (NEFA) at the end of the study.

Plasma insulin was measured by radioimmunoassay (Coat- A-Count Insulin RIA kit; Diagnostic Products Corporation, California, USA), and serum non-esterified fatty acids (NEFA) by enzymatic colorimetry (NEFA-HA, Wako Ltd, Osaka, Japan). Area under the curve for the resulting glucose and insulin response profiles was calculated using the trapezoidal rule [635], and insulin resistance and β-cell function determined using the homeostasis model assessment 2 (HOMA2) (HOMA2 Calculator, University of Oxford, Oxford, UK) [636].

Percentage insulin stimulated NEFA suppression at 30 and 60 minutes were calculated by [637]:

$$NEFA_{sup30}\,\% = (NEFA_0\text{-}NEAF_{30})/NEFA_0$$

$$NEFA_{sup30}\,\% = (NEFA_0\text{-}NEAF_{60})/NEFA_0$$

where the subscript *0*, *30*, and *60* indicate baseline, 30 minute and 60 minute values, respectively. NEFA suppression was calculated from samples taken during the OGTT only. However, fasting NEFA as reported below is the average of two fasting NEFA results obtained on non-consecutive days preceding the OGTT and submaximal exercise test, respectively.

5.2.6 Substrate Utilisation at Rest and during Exercise

Resting metabolic rate (RMR) was determined by having participants lie supine for 30 minutes while respiration and haemodynamic parameters were monitored. Respiration was monitored as noted above for the maximal progressive exercise test. Haemodynamic monitoring is described below. Data from 10-25 minutes were used to reflect a true resting state. Then, still in a fasted state, participants cycled for 60 minutes at 50% of the resistance corresponding to VO_{2peak} achieved during their screening maximal progressive exercise test. Respiration was monitored for five minute periods every 15 minutes, and a blood sample collected.

The respiratory exchange ratio (RER) was used to determine substrate utilisation, and blood samples to determine concentrations of glucose, insulin, and NEFA, as described above. RER was calculated:

$$RER = VCO_{2\ out}/VO2_{in}$$

where VCO_2 and VO_2 indicate volume of carbon dioxide and oxygen, respectively, in L/min, and the subscripts *out* and *in* indicate gas eliminated and gas consumed, respectively.The averages from fifteen minutes of supine rest and the average of the four five minute respiratory measurements were used to determine RER at rest and during submaximal exercise, respectively. For the resting measure, patients rested for five minutes before sampling, and during exercise measures taken in the first minute were discarded to ensure a steady measure.

5.2.6 Resting Heart Rate and Blood Pressure

Haemodynamics at rest were measured using bioimpedance (Task Force® Monitor, CNSystems Medizintechnik GmbH, Graz, Austria), specifically: systolic and diastolic, and heart rate.

5.2.7 Body Composition and Anthropometry

Body composition and anthropometry were assessed as described in *Section 4.2.7.1* and *Section 4.2.7.2*, respectively.

5.2.8 Intervention

Control participants were asked to maintain their current physical activity habits and treatment plans including all medication. All participants were instructed to retain their current diets, physical activity levels outside of the intervention, and body weight. To assist with weight stability during the intervention, participants were asked to report their weight on a biweekly basis. If weight change exceeded 1% of baseline weight, the participant was instructed to modestly increase or decrease their food intake until their weight approached that at baseline.

Participants in the HIIT group were instructed to complete a cycle ergometer-based training protocol three times per week, where feasible on non-consecutive days, for twelve weeks. Intensity was based on the 6-20 point Borg Rating of Perceived Exertion (RPE) [638]. Participants were provided with a laminated A4 sheet depicting the RPE scale and a picture guide for exercises (see *Appendix 1*), and an iPod shuffle (Apple Inc. Silicon Valley, CA, USA) containing pre-recorded instructions and verbal cues to guide them through each session (see *Appendix 9*). Sessions consisted of a 5 minute warmup progressing from an RPE of 9 (*very light*) to 13 (*somewhat hard*) followed by five intervals of cycling at an RPE of16-17 (*very hard*) interspersed with three minute recovery periods, and followed by a 3 minute cool-down after the last interval. Each interval was two minutes long in the first week with 10 seconds added per week, so that intervals were three minutes and 50 seconds long by week 12. The programme therefore took 30 to 40 minutes to complete depending on week. Recovery periods included 90 seconds of passive

recovery, 60 seconds of band resisted upper body exercise, and 30 seconds to transition back onto the ergometer, start cycling, and programme the required settings for the upcoming interval. One upper body exercise was performed per recovery period in the following order: face-pull; horizontal push; horizontal pull; and 45º push (see *Appendix 1*). Adequate adherence was defined as self-reported completion of at least 33 exercise sessions. This was corroborated by an exercise diary in which date and maximal heart rate of each session were recorded by each participant.

5.2.9 Statistical Analysis

Numeric data was recorded in Excel® 2008 for Mac (Microsoft Corporation, Silicon Valley, USA.) and analysed using SPSS version 19 (IBM, New York, USA). Prior data indicated that the standard deviation of liver fat in a population similar to that of the study was between 7.4 and 9.1 [243]; thus, assuming a standard deviation of 9.0, an 80% chance of detecting a 10% relative change in liver fat concentration could at a one-sided 0.05 significance required n=22. A target n=28 was chosen to allow for 20% dropout.

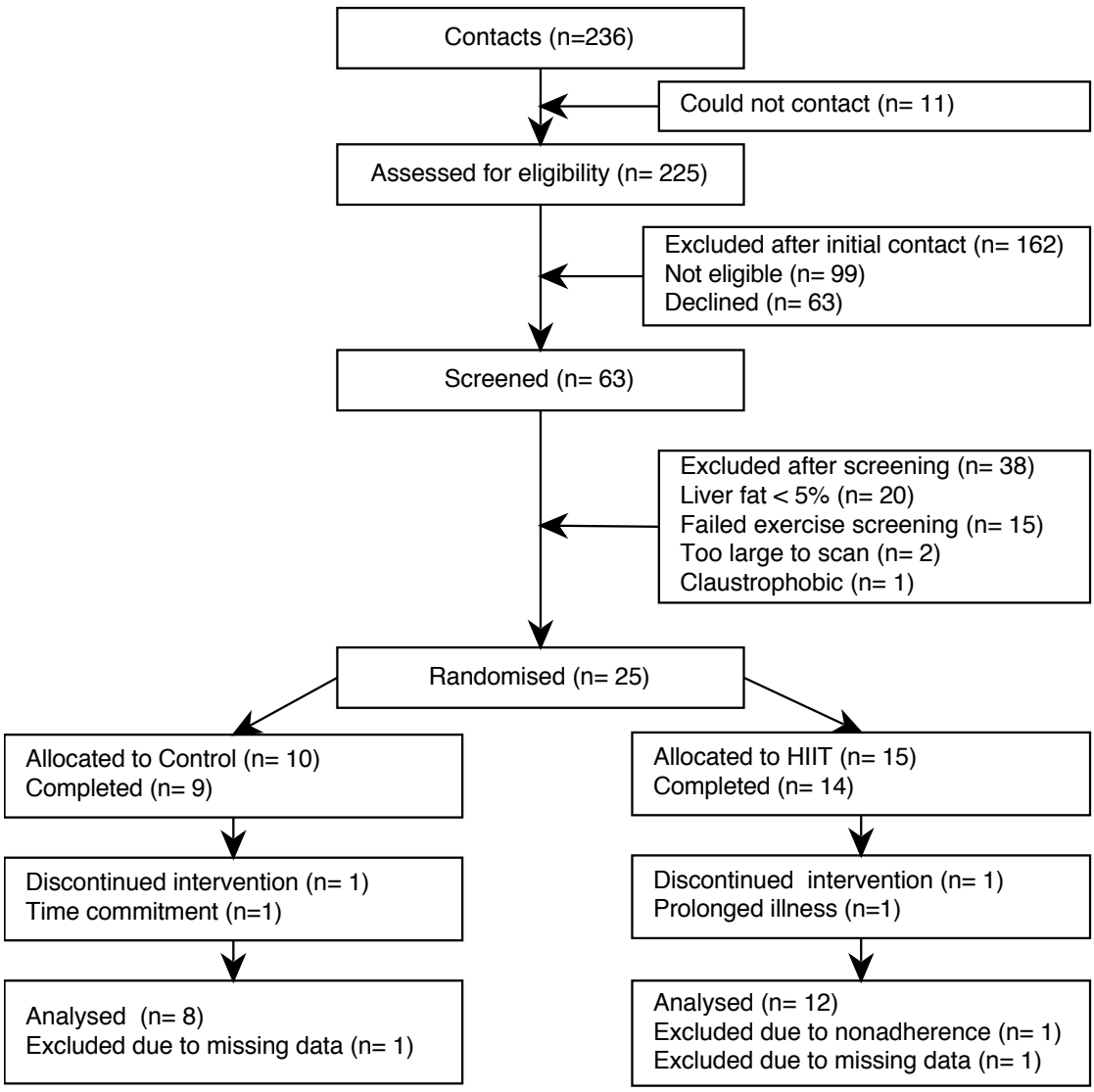
Statistical analysis was per-protocol as this was a phase II trial to establish efficacy not effectiveness. Normality of data distribution was established by tests of skewness and kurtosis. Where data were non-normally distributed these were transformed by their natural logarithm to achieve normality and retested. Between group comparisons were made using analysis of covariance with the followup result as the dependent variable and the baseline result as the covariate. Because liver fat is particularly responsive to weight change, a model including weight change and one including change in total fat mass were also run. Comparisons of key baseline variables (see *Table 5.1*) were made using independent sample T-Tests. Within group changes were assessed by paired-sample T-Test. Given not all data were normally distributed, Spearman rank correlation coefficient were used on untransformed data to test the association of IHTAG and two hour glucose with changes in body composition and baseline fitness.

5.3 Results

5.3.1 Baseline Comparisons and Participant Flow

Participant flow is shown in *Figure 5.1*. Of 25 people randomised, 20 people were analysed: one male withdrew from the control group, and one female was excluded due to key missing data; one female withdrew from the HIIT group due to prolonged illness, one female did not complete the minimum number of exercise session, and one was excluded due to key missing data. There were no study related serious adverse events.

Figure 5.1: CONSORT Statement Flow of Participants



Key baseline data are shown in *Table 5.1.* There were more females in the HIIT than the CON group. Age, BMI, weight, height, peak oxygen consumption, and peak work rate were similar between groups. Based on 2-hour glucose and no prior diagnosis, no controls had type 2 diabetes and one male had impaired glucose tolerance, whereas in the HIIT groups one female had type 2 diabetes, and three females and two males had impaired glucose tolerance. Prescription medication used by participants is shown in *Table 5.2*.

Table 5.1: Baseline Characteristics

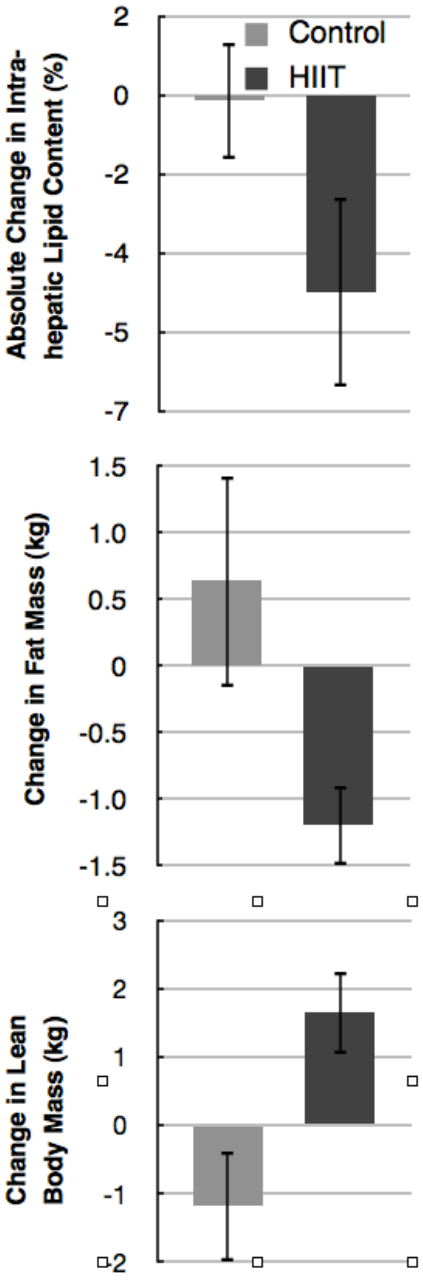
Parameter	Control	HIIT	*p*
N (males/females)	8 (7/1)	12 (7/5)	
Age (years)	49.8 (42.7, 56.8)	52.9 (47.5, 58.4)	0.499
BMI (kg/m^2)	31.1 (27.3, 34.9)	30.3 (27.9, 32.8)	0.731
Weight (kg)	90.0 (82.7, 97.3)	86.7 (78.7, 94.8)	0.562
Height (m)	1.71 (1.64, 1.78)	1.69 (1.65, 1.73)	0.650
VO_2peak (L/min)	2.3 (1.9, 2.6)	1.9 (1.4, 2.3)	0.157
VO_2peak (ml/kg/min)	24.9 (21.2, 28.6)	22.5 (19.2, 25.8)	0.363
VO_2peak (ml/kg$_{\text{lean mass}}$/min)	38.1 (34.6, 41.7)	33.7 (28.9, 38.5)	0.164
Peak work rate (W)	162 (137, 187)	144 (119, 170)	0.341

Table 5.2: Prescription Medication

Medication (Class)	Control (n)	HIIT (n)
Statin		2
Diuretic		2
Selective seratonin reuptake inhibitor		1
Bisphosphonate (osteoporosis)		1
β-agonist (asthma)		2
Antihistamine/allergy medication		2
Analgesics	1	
Topical steroid anti-inflammatory		1

5.3.2 Liver Fat and Body Composition

Liver fat and body composition changes are shown in *Table 5.2*. Between group comparisons showed significant reductions in IHTAG, fat mass and body fat percentage, and an increase in lean body mass in HIIT vs. controls by (mean ±SD) 4.4±7.4%, 1.6±1.1 kg, and 1.2±1.4%, and 1.0±1.9 kg, respectively (see *Figure 5.2*). Adding weight change as a covariate did not change the significance of the finding (p= 0.03), but using change in total fat mass did negate statistical significance (p= 0.082). There were no significant correlations between changes in IHTAG and changes in body weight, fat mass, fat free mass, or baseline VO_{2peak}.



Within group comparisons showed a significant reduction in weight, BMI, fat mass, and body fat percentage in HIIT. However, IHTAG reduction and fat free mass increase were not significant in the within group comparison (p= 0.066, and p= 0.095, respectively). IHTAG increased in 6 controls and decreased in two, whereas it increased in only one HIIT and decreased in the other 11.

Visceral fat areas remained similar pre and post intervention in both groups. Subcutaneous fat area was not analysed as there were technical problems with several images such that interrater error on many images would have exceeded the actual pre vs. post differences observed in some scans.

Table 5.3: Changes to Body Composition and Fat Distribution

Parameter	Control		HIIT		*p* treatment x time
	Baseline	Post	Baseline	Post	
IHTAG($_{^1H\text{-}MRS}$) (%)	10.1 (7.0, 13.2)	10.0 (5.1, 8.1)	10.9 (7.2, 14.7)	6.6 (5.1, 8.1)	*0.031*
Weight (kg)	90.0 (82.7, 97.3)	89.9 (83.2, 96.5)	86.7 (78.7, 94.8)	85.6 (77.6, 93.6)†	0.149
BMI (kg/m^2)	31.1 (27.3, 34.9)	31.1 (27.3, 34.9)	30.3 (27.9, 32.8)	29.9 (27.5, 32.3)†	0.162
Fat mass (kg)	31.0 (24.1, 37.8)	31.5 (24.3, 38.6)	32.8 (28.3, 37.4)	31.2 (26.6, 35.8)‡	*0.018*
Body fat (%)	34.0 (28.3, 39.7)	34.6 (28.2, 41.1)	37.5 (34.1, 40.9)	36.3 (32.7, 39.9)*	*0.024*
Fat free mass (kg)	59.0 (54.2, 63.8)	58.4 (52.9, 64.0)	53.9 (48.4, 59.4)	54.9 (48.8, 61.0)	*0.032*
Visceral (cm^2)	150 (117, 183)	145 (120, 170)	147 (122, 173)	149 (117, 181)	0.520

Data are shown as means and 95% confidence intervals. Abbreviations: ^{1}H-MRS, proton-energy magnetic resonance spectroscopy; IHTAG, intrahepatic triacylglycerol.
* significantly different from baseline within group $p< 0.05$
† significantly different from baseline within group $p< 0.01$
‡ significantly different from baseline within group $p< 0.001$

5.3.3 Metabolic Changes

Changes to glucose, insulin, NEFA, and energy substrate utilisation are shown in *Table 5.3*. There were no significant between-group changes in fasting or 2-hour glucose, glucose area under the curve, or HbA_{1c}. There was a significant within-group reduction in 2-hour glucose for HIIT. There were no within or between group differences in respiratory exchange ratio at rest or during exercise.

5.3.4 Liver Enzymes, Blood Lipid Fractions, and Serum Ferritin

Key liver enzymes, blood lipids, and serum ferritin results are shown in *Table 5.4*. Both within and between group comparisons indicated a significant reduction of ALT and AST, but only between group comparison showed a reduction in TAG concentrations. Within group comparisons showed a reduction in serum ferritin in both controls and HIIT.

Table 5.4: Metabolic Changes

Parameter	Control		HIIT		*p* treatment x time
	Baseline	Post	Baseline	Post	
Fasting glucose (mmol/L)	4.7 (4.5, 5.0)	4.7 (4.4, 5.0)	4.7 (4.5, 5.0)	4.7 (4.5, 4.0)	0.771
Fasting insulin (pmol/L)	84 (64, 104)	87 (69, 105)	125 (92, 157)	100 (72, 127)	0.205
Fasting NEFA (mmol/L)	0.46 (0.36, 0.57)	0.54 (0.43, 0.65)	0.49 (0.39, 0.59)	0.50 (0.42, 0.58)	0.179
2 hour glucose (mmol/L)	5.8 (4.9, 6.8)	5.4 (4.5, 6.2)	8.1 (6.6, 9.6)	6.6 (5.6, 7.5)*	0.799
2 hour insulin (pmol/L)	712 (250, 1173)	884 (473, 1294)	1080 (768, 1391)	1054 (765, 1344)	0.979
fsOGTT glucose AUC	876 (788, 965)	896 (836, 956)	989 (883, 1095)	923 (828, 1019)	0.317
$NEFA_{suppression\ 30\ minutes}$ (%)	5.5 (-12.9, 23.9)	-7.9 (-19.5, 3.8)	7.4 (-6.7, 21.5)	-2.8 (-19.8, 14.2)	0.679
$NEFA_{suppression\ 60\ minutes}$ (%)	17.4 (-5.0, 39.9)	5.4 (-3.0, 13.9)	6.3 (-14.3, 26.9)	6.1 (-18.1, 30.2)	0.884
HOMA2-IR	1.6 (1.2, 1.9)	1.6 (1.3, 1.9)	2.2 (1.7, 2.8)	1.8 (1.3, 2.3)*	0.229
HOMA2-β	142 (114, 170)	152 (124, 180)	183 (152, 215)	156 (130, 182)	0.200
HOMA2- S	72 (54, 90)	69 (53, 85)	55 (38, 72)	70 (46, 94)	0.209
HbA_{1c} (%)	5.5 (5.2, 5.8)	5.6 (5.3, 5.8)	5.9 (5.6, 6.2)	5.9 (5.7, 6.2)	0.913
$RER_{resting}$	0.87 (0.82, 0.92)	0.91 (0.86, 0.96)	0.90 (0.87, 0.93)	0.88 (0.85, 0.92)	0.259
$RER_{submaximal\ 10\text{-}15\ minutes}$	0.98 (0.94, 0.99)	0.97 (0.94, 1.00)	0.99 (0.96, 1.02)	0.98 (0.93, 1.03)	0.758
$RER_{submaximal\ 25\text{-}30\ minutes}$	0.94 (0.92, 0.94)	0.94 (0.91, 0.94)	0.95 (0.92, 0.99)	0.96 (0.91, 1.01)	0.682
$RER_{submaximal\ 40\text{-}45\ minutes}$	0.92 (0.90, 0.95)	0.92 (0.90, 0.95)	0.95 (0.92, 0.98)	0.95 (0.92, 0.98)	0.270
$RER_{submaximal\ 55\text{-}60\ minutes}$	0.92 (0.89, 0.94)	0.91 (0.90, 0.93)	0.93 (0.90, 0.96)	0.94 (0.91, 0.98)	0.281
$RER_{submaximal\ mean}$	0.93 (0.91, 0.96)	0.94 (0.91, 0.96)	0.95 (0.92, 0.99)	0.96 (0.92, 1.00)	0.529

Data are shown as means and 95% confidence intervals. Abbreviations: AUC, area under the curve; fsOGTT, frequently samples oral glucose tolerance test; HBA1c, glycated haemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment of beta cell function; ISI, insulin sensitivity index; NEFA, non-esterified fatty acids, RER, respiratory exchange ratio.

* significantly different from baseline within group $p < 0.05$

† significantly different from baseline within group $p < 0.01$

‡ significantly different from baseline within group $p < 0.001$

Table 5.5: Changes to Liver Enzymes and Blood Lipids

Parameter	Control		HIIT		*p* treatment x time
	Baseline	Post	Baseline	Post	
ALT (IU/L)	48 (31, 65)	53 (33, 72)	59 (39, 79)	43 (28, 58)*	*0.019*
AST (IU/L)	31 (24, 38)	35 (26, 45)	39 (27, 51)	33 (24, 42)*	*0.023*
GGT (IU/L)	48 (18, 78)	56 (14, 98)	55 (9, 100)	38 (10, 66)	0.061
Serum ferritin (µg/L)	166 (133, 200)	142 (111, 173)*	110 (65, 154)	78 (43, 113)†	0.065
Total-cholesterol (mmol/L)	5.6 (4.8, 6.5)	5.5 (4.6, 6.5)	5.2 (4.5, 5.8)	5.0 (4.5, 5.6)	0.594
LDL-cholesterol (mmol/L)	3.8 (3.1, 4.5)	3.6 (2.8, 4.3)	3.2 (2.7, 3.8)	3.1 (2.6, 3.6)	0.842
HDL-cholesterol (mmol/L)	1.1 (1.0, 1.2)	1.2 (1.0, 1.4)	1.1 (0.9, 1.3)	1.1 (0.9, 1.3)	0.452
Total: HDL-cholesterol	5.0 (4.5, 5.5)	4.9 (4.4, 5.5)	4.9 (4.0, 5.8)	4.7 (3.9, 5.6)	0.617
Triacylglycerols (mmol/L)	1.5 (1.1, 2.0)	1.8 (1.2, 2.5)	1.8 (1.4, 2.1)	1.6 (1.3, 2.0)	*0.047*

Data are shown as means and 95% confidence intervals. Abbreviations: ALT, alanine aminotransferase; AST , aspartate aminotransferase; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
* significantly different from baseline within group $p < 0.05$
† significantly different from baseline within group $p < 0.01$

5.3.5 Changes to Cardiac and Haemodynamic Parameters

Resting heart rate, and systolic and diastolic blood pressure are shown in *Table 6.6*. There were no changes over time within or between groups.

Table 5.6: Heart Rate and Blood Pressure

Parameter	Control		HIIT		p treatment x time
	Baseline	Post	Baseline	Post	
HR_{rest}	65 (60, 71)	67 (61, 73)	65 (61, 69)	63 (60, 65)	0.068
SBP_{rest} (mmHg)	123 (118, 127)	124 (119, 130)	131 (124, 137)	128 (118, 139)	0.540
DBP_{rest} (mmHg)	83 (79, 88)	86 (80, 91)	89 (84, 95)	88 (81, 94)	0.595

Data are shown as means and 95% confidence intervals. Abbreviations: BP, blood pressure;

5.4 Discussion

NAFLD affects a large portion of the World's adult population and has several hepatic and non-hepatic sequelae including NASH, fibrosis, cirrhosis and hepatocellular carcinoma on the one hand, and type 2 diabetes and cardiovascular disease on the other (see *Section 1.8*). Lifestyle therapy focusing on weight reduction is the preferred, and in the absence of approved pharmacological interventions, only means to address NAFLD [14,311]. However, weight reduction and subsequent maintenance is difficult for many patients even when enrolled in research interventions [358]. Exercise only interventions may offer an alternative independent of weight reduction, but are underrepresented in the literature, potentially leading them to be ignored as an option in clinical practice (see *Chapter 2*).

This is the first study to assess the effects of HIIT in patients with clinically defined NAFLD. The major findings are that HIIT performed three times per week for 12 weeks led to: 1) a 40%reduction in IHTAG; 2) improved body composition; 3) and reduced plasma ALT and AST; and 4) improved glucose control. The observed 40% relative reduction in IHTAG was greater than the 10-21% previously reported following resistance or aerobic exercise only interventions [243-245], and comparable to several weight reduction trials (see *Chapter 2*). The the greater IHTAG reduction relative to other exercise based interventions may be partly accounted for by the present study having a greater duration than some [243,244], but not all previous studies [245]. Although there may have been a difference in exercise related energy expenditure, this was not measured in the present study. The best explanation for the reduction in liver fat was the notable reduction in body fat mass suggesting increased fat oxidation.

Notably, the present study did not show a significant change in body weight in the HIIT group vs. controls; the within group analysis indicated a very modest 1 kg weight reduction following exercise. This corroborates the findings of previous exercise interventions that have shown IHTAG can be reduced in the absence of marked body weight reduction [243-245]. The minor weight change observed in the HIIT group was consistent with similar modest weight reductions observed in broadly comparable HIIT trials (see *Section 3.3.3*).

However, patients in the present study were specifically asked to monitor and maintain their weight. The HIIT intervention did, however, elicit a significant change in body composition. The observed reduction in fat mass is consistent with that reported by some, but not all other studies employing an HIIT protocol over 12 or more weeks (see *Section 3.3.3*). Modest increases in fat free mass have also been previously reported [494], interestingly in the context of a reduced calorie diet.

Lean body mass is inversely associated with mortality [573,639,640], and loss of lean mass is associated with insulin resistance [641], especially in older populations. Resistance training has previously been shown to promote retention of lean mass during weight reduction [642-645]. The finding of the present study suggests HIIT may be another means by which to promote maintenance of lean mass during weight reduction.

Interestingly, HIIT produced a change in IHTAG without any apparent change in visceral fat area. There is increasing evidence that the two depots tend to reflect adiposity and are not mechanistically linked [646]. Recent findings from the Framingham Heart Study show IHTAG to be associated with the dyslipidaemia and dysglycaemia independently of visceral fat [622]. The observation of decreased IHTAG in the absence of any observable change in visceral fat in the present sample, provides further information on the separate regulation of IHTAG and visceral fat. However, given the modest reductions in total fat, any change in visceral fat volume may have been too small to detect using single slice analysis [647].

Although no previous studies have directly reported the effects of HIIT on IHTAG, HIIT has been shown to reduce hepatic secretion of very-low density lipoprotein triacylglycerol [504], and reduced postprandial plasma triacylglycerol concentrations [82,83], indicating a direct effect on hepatic metabolism. Indeed, there was a relative reduction in fasting plasma triacylglycerol in the present HIIT group.

Glucose control and insulin sensitivity as assessed by frequently sampled oral glucose tolerance test, HOMA-IR, NEFA suppression, and HbA_{1c} concentration did not following HIIT relative to control conditions. There was a notable within group decrease in two-hour glucose concentration in the HIIT group. These finding were contrary to some though not all previous reports of the effect of HIIT on glucose control (see *Section 3.3.4.21*). Further, the ability of aerobic exercise to transiently improve glucose control by increasing both insulin dependent and insulin independent myocellular glucose uptake is well documented [64].

There are a number of factors that are likely to have influenced the present results. Most notable are the transient nature of the metabolic changes following exercise, and the heterogeneity of the cohort with respect to degree of insulin resistance. A cumulative effect of exercise on glucose clearance has been observed [104]. However, even in endurance trained athletes with high insulin sensitivity, three days of detraining resulted in one third reduction in rate of glucose clearance, and seven days of detraining resulted in glucose clearance similar to that of sedentary controls [593]. In a previous HIIT cohort, reductions in area under the insulin curve and improvements in the insulin sensitivity index were observed 24 but not 72 hours post training [479]. This is consistent with observations following classic continuous moderate intensity aerobic exercise where insulin sensitivity has been shown to stay elevated for 48 hours but not 5 day [592]. Further, high carbohydrate intake in the hours immediately following exercise has been shown to blunt the insulin sensitising effect of exercise indicating that some degree of glycogen depletion is required [508].

In the present study volunteers were asked to refrain from exercise 48 hours prior to assessments to avoid observing changes exclusively due to the last bout of exercise, however in practice the period of time between exercise and metabolic assessments ranged between 48 hours and several days. Further, no data on post exercise dietary practices were collected. Although HbA_{1c} is considered a longer-term marker of recent glucose control and not likely to be measurably affected by a few days of detraining, most participants had concentrations already within the normal range at baseline. Future

investigations would benefit from using techniques such as continuous glucose monitoring to obtain a more longitudinal data on the effect of exercise on glucose control.

Energy substrate utilisation during rest and sub-maximal exercise showed no change within or between groups as assessed by RER. Two previous studies reported no change in resting RER 48 [504], and 72 hours post-exercise following 2 and 8 weeks of HIIT [505], respectively. Whereas one 12 week HIIT intervention did result in a 2.4% reduction in RER indicating increased fat oxidation and decreased carbohydrate oxidation, however the time elapsed since the last bout of exercise was unreported [492]. An approximate 65% increase in fat oxidation and 20% reduction in carbohydrate metabolism has been reported 60 minutes post exercise [501]. This was already evident after two weeks of training, with no further changes at six weeks.No reports of energy substrate utilisation during submaximal exercise following an HIIT programme were found in the literature. Given the transient nature of changes to glucose metabolism following exercise, it is feasible that any change that might take place following exercise was lost within the period between exercise and post-intervention assessment in both the present and above studies.

HIIT has established itself as both time efficient and effective at substantially improving cardiorespiratory fitness in a broad range of clinical and healthy populations (see *Chapter 3*). Increased fitness is desirable given the inverse relationship between participation in vigorous exercise and progression from uncomplicated steatosis to NASH [240]. Time efficiency may be also be critical as a lack of time is a frequently cited barrier to regular exercise [648]. A HIIT approach was also preferred over continuous moderate intensity exercise by obese women with and without type 2 diabetes [459], and coronary heart disease patients [449].

Metabolic adaptations following HIIT are comparable and sometimes greater than those following prolonged continuous moderate intensity exercise [649]. Single [420], and multiple sessions of HIIT have been shown to improve 24-hour and postprandial glucose control in type 2 diabetes [421]. HIIT over a number of weeks also improved glucose control in people with metabolic syndrome [503].

Muscle biopsies following HIIT in both healthy individuals and those with type 2 diabetes indicate these changes are at least partly accounted for by increased mitochondrial biogenesis via an intensity dependent mechanism, and include increased expression of glucose transporter and elevated resting glycogen in muscle [649].

This study had multiple characteristics that can be viewed as either strengths or limitations. The cohorts were small and, despite randomization, somewhat heterogeneous with respect to some biochemical, anthropometric, and cardiac parameters. The presence of significant fibrosis was all but ruled out using the NAFLD fibrosis score, so the present cohort was most representative of the majority of NAFLD patients seen in primary care. All but the first 1-2 exercise sessions were unsupervised and program adherence was only assessed by self-report. This reflects the likely scenario in a clinical setting, but does not optimize adherence. Further, the nature of the assessments required they be conducted over a minimum of two days, three for most volunteers, and no volunteers were able to do them consecutively due to other commitments. Although the timeframe of IHTAG change following exercise is not known, exercise induced improvements in glucose tolerance and insulin sensitivity, although cumulative over several exercise sessions [104], are most marked within the first 24-48 hours post exercise, and tend to decline markedly after 72 hours even in athletes [479,591]. Thus efficacy of the HIIT protocol done as prescribed, may have been underestimated due to delays in assessment following completion of the intervention.

Assessment of outcomes was by high quality objective, and extensively validated methods, specifically: ^{1}H-MRS to quantitatively and objectively assess liver fat; MRI to quantify abdominal fat; air displacement plethysmography to assess body composition; frequently sampled oral glucose tolerance test to assess multiple aspects of glucose control and insulin response; and indirect calorimetry to assess metabolism during rest and submaximal exercise.

5.5 Conclusion

A thrice weekly programme of unsupervised progressive HIIT lasting 30-40 minutes per session markedly reduced liver fat, decreased ALT and AST, improved body composition, and reduced two-hour glucose with minimal change in total body weight in adults with NAFLD. This exercise programme therefore represents a time efficient means to improve the health of patients with NAFLD, particularly those unable or unwilling to adopt lifestyle changes resulting in weight reduction.

Chapter 6. Limitations of the Research in this Thesis

This chapter summarises the limitations of the primary and secondary research that form the basis of this thesis, and notes how these might be addressed in future work.

6.1 Limitations of Chapter 2

The systematic review of in Chapter 2 was the first of of its kind done assessing lifestyle therapy in NAFLD at the time it was published [15]; although there was already a systematic review looking at weight reduction in NAFLD [336]. Applying a systematic approach to reviewing lifestyle interventions in NAFLD was justified on the basis that such an approach is required to determine gaps in knowledge and also in the quality of the evidence base on which that knowledge is based.

Chapter 2 shares the key limitation of all systematic reviews in that it can only be as good the research it seeks to summarise, which as noted in Chapter 2 is of limited quality. Perhaps not sufficiently emphasised is the absence of long-term follow-up in the studies reviewed. Therefore any inference about the effect of the interventions reviewed on NAFLD prognosis must be made very tentatively until intervention studies employing large cohorts with multi-year follow-up and hard disease end-points can be included. Further, the review did not include meta-analytic techniques as study and intervention design was considered too heterogenous to justify such analysis. Future reviews should seek to pool data from relevant studies, as appropriate, to provide a more qualitative assessment of both effectiveness and efficacy, as well as applying regression techniques to assess dose response for factors such as: active intervention length; degree of weight reduction; degree of body composition change; and in the case of exercise also type, frequency, duration, and intensity.

A further specific limitation of the review was the exclusion of non-english language papers. Including foreign language papers may have strengthened the review and should be included in future reviews.

As noted in the discussion section of Chapter 2, a common limitation of systematic reviews is that they focus on specific tightly defined populations, in this case cohorts with established NAFLD. This is necessary to make it clear to whom results can be extrapolated. However, there are several practical limitations when applying a rigorous population definition:

- Disease genotype and phenotype can still be highly heterogenous even if all members of a cohort have been diagnosed with a specific condition;
- There are multiple criteria by which the presence of NAFLD is assessed and these are not interchangeable;
- There several published interventions in cohorts with common comorbidities of NAFLD, notably metabolic syndrome and type 2 diabetes, which are likely to contain a high proportion of participants with NAFLD. Notably, many of these interventions such as the diabetes prevention trials and Look AHEAD study are large scale and have many years of followup. These were however only considered in the discussion section of Chapter 2 and not at all in the published review [15]

Therefore including larger longer term interventions in populations with common comorbidities of NAFLD may have strengthened the conclusions of the review by providing higher levels of evidence and statistics on hard endpoints.

Future systematic reviews would benefit from an attempt to focus on integrating the findings from high quality interventions with long-term followup focusing on primary or secondary prevention in cohorts with or at high risk of common metabolic disease; specifically NAFLD, metabolic syndrome, and/or type 2 diabetes.

6.2 Limitations of Chapter 4

The Physical Activity, Ageing and Metabolism (PAAM) study had several notable limitations:

- the sample size was small, leaving the study underpowered
- restricting the study to women introduced the potential for results to be influenced by:

    - menstrual status and time during cycle;
    - oral contraceptive use;
    - parity;
    - use of hormone replacement therapy
- the study was cross-sectional

The PAAM study was a pilot study to help establish requirements for a larger investigations. Assuming regression analysis is used for statistical assessment, the effect size could be used to calculate power. Based on the findings, assuming two-sided significance (1-alpha) of 95%, a desired power (1-beta) of 80, with an equal ratio of active and sedentary women, and assuming a small effect size of 0.10 for primary outcome measures with two predicators (age and activity), the minimum sample would be 99 in a given age group.

The choice of restricting the study to women was made due to the more limited published data in females relative to males with respect to ageing, physical activity and metabolism. Confounders related to hormonal cycles as noted above would best be addressed by either a large sample size and the collection data on menstrual phase, use of oral contraceptives, parity, time since menopause, and use of hormone replacement therapy. These factors could then be added as components of statistical regression models to determine the size of their influence. This would increase the sample size required as it would add to the number of predictors in the model.

Consideration should also be given to the need for longitudinal research assessing the relationships between physical activity and metabolism using the methods employed in the PAAM study, e.g. accelerometry, are justified.

6.3 Limitations of Chapter 5

The high-intensity intermittent training intervention had the following limitations:

1. the final analysis included fewer participants than required by the power calculation leaving the study underpowered;

2. numbers of participants in the control vs. exercise intervention were uneven which may have contribute to the following:
    3. there was a notably uneven sex distribution between the control group and exercise intervention group (1 vs. 5);
    4. there was also a significant difference between groups in terms of 2-hour glucose and insulin, and HOMA2-insulin resistance;
5. programme adherence was only assessed by self-report weakening the assessment of efficacy;
6. post-intervention assessments were not standardised in terms of time expired since last exercise bout, thereby potentially leading under or over estimation of effect.

Future assessments of high-intensity intermittent exercise, and any other forms of exercise, should be sufficiently powered for all end-points considered important. Further, as this is still likely to result in small sample sizes, randomisation should be stratified by sex and primary outcome measure(s) to improve similarity between control and intervention groups.

Optimal assessment of adherence with the exercise protocol would require all sessions to be supervised and conducted on equipment that allows measurement of work rate. Adherence could have been more objectively assessed by means of accelerometery or heart rate monitoring to assess physical activity, or by using GPS tracking devices or reviewing gym attendance databases to confirm attendance; the former options would be preferable as they allow assessment of exercise intensity.

It will be important to standardise the timing of post-intervention assessments so that they are consistent with the exercise prescription, so that the average effects are neither under nor overestimated. This could be best achieved by planning the post-intervention assessment during the final week of intervention so that all assessments could be done within 48-72 hours of an exercise session.

There are further limitations with respect to determining effectiveness:

- the sample size was small;
- the intervention was for a short time with no interim assessments conducted, thus the study cannot provide information on the time-course of changes nor does it answer what the changes might be observed over longer periods. there was selection bias as patients had to volunteer to be given the exercise prescription as opposed to having it provided as part of standard care;
- the study was confined to volunteers in who the probability of significant fibrosis was small, thus limiting extrapolation to relatively low risk patients.

The study was not intended to assess effectiveness in a clinical setting, nor to assess exercise in patients with more advanced NAFLD. It was designed to provide physiological data on changes following high-intensity intermittent training in a group representative of the majority of primary care NAFLD patients.

To address the effectiveness in a clinical setting a much larger study would need to be conducted; preferably including participants with more advanced liver disease. Treatment allocation in such a study would best be done by block randomisation on the basis of site with a multi-year followup, and an intention to treat analysis.

Such a study would also need to employ behaviour change techniques to maintain exercise habits over a long period. Assessment of hard endpoints including NAFLD disease progression by biopsy or validated surrogates, development or regression of impaired glucose metabolism by HbA1c and potentially oral glucose tolerance tests, and tracking development of cardiovascular disease.

A sample size allowing for subsample analysis, e.g. by sex and genotype, would be desirable, as this would allow the identification of those most likely to benefit

from the exercise programme. Similarly,assessments at multiple time-points would allow the time-course of changes to be determined.

Chapter 7. Discussion

Non-communicable diseases including type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), cardiovascular disease, and some cancers are rising in prevalence worldwide [650]. In 2008, 25% of deaths in those under 60 years of age were attributable to non-communicable diseases [651]. Cardiovascular disease and diabetes combined accounted for 13.2% of disability adjusted life years in high income countries in 2004 [652]. The worldwide financial burden of cardiovascular disease and diabetes was estimated at £539 billion and £312 billion, respectively, and is predicted to rise to £650 billion and £465 billion, respectively, by 2030 [650].These costs are threatening to overwhelm many healthcare systems and erode the quality of life of large portions of the population including in the United Kingdom [12].

This thesis set out to examine these issues of deteriorating population health and explore potential lifestyle related solutions through the lens of NAFLD and impaired glucose control. Specifically, it set out to assess: the relationship of age and physical activity with liver fat and glucose control (Chapter 4); the efficacy of diet, weight management, and exercise in addressing markers of liver health and glucose control in those with NAFLD (Chapter 2); and examine the potential for high-intensity intermittent training as a modifier of metabolism and therefore potential therapy for NAFLD and impaired glucose tolerance (Chapters 3 & 6).

There is a close association of liver health and glucose control with age, and physical (in)activity is a key modifier of this relationship (Chapter 4). Increasing population age [653], the rising prevalence of overweight/obesity [654], and a predominance of sedentary behaviour [655], appear central to the rise in chronic disease burden [518,654], including the high prevalence of NAFLD [5]. Advancing age is associated with a decline in physical activity and adverse changes in body composition in the form of decreasing lean mass and increasing fat mass (*see Sections 4.4.1*). Likewise, physical inactivity is associated with an increase in fat mass and, throughout much of the lifecycle, increased total body weight (*see Sections 4.4.1*). Despite these close associations, the results in Chapter 4

suggest these relationships are not entirely causal, nor sequence of events inevitable. The decline in physical activity and increase in obesity with age is influenced by a population-wide upward trend in sedentariness and overweight/obesity evident since the early to mid 1990s [567,656]. Likewise, the prevalence of many chronic diseases and elevated disease risk factors is increasing in all age groups, not just the elderly [567,576,657]. Further, those over 90 years often display a favourable cardiometabolic risk profile [585], highlighting the importance of cardiometabolic health in achieving lifelong health and wellbeing.

The environmental changes associated with declining population health include the emergence of numerous labour saving devices and automated transport with associated marked decline in work and transport related physical activity [658,659], and changes to diet presumably driven by the changes in food availability and practices surrounding food processing and preparation [659]. These changes can be, and to some extent are being, addressed on the legislative and policy level in the hopes of reducing the burden of chronic disease. General as well as disease specific guidelines stress the benefits of physical activity [416], and weight management [660]. The findings of this thesis support such guidelines, but highlight that a broad variety of dietary and physical activity approaches may be therapeutic than are presently advocated (Chapter 2).

With respect to diet, although Chapter 2 highlights the utility of energy restricted moderate-to-low fat approaches to weight reduction for lowering liver fat, the studies reviewed also highlight other potential approaches. Carbohydrate restriction appears to effectively and rapidly reduce liver fat and liver glucose production, with notable improvements arising before substantial weight reduction is achieved [171]. One or more weight change independent mechanisms of liver fat reduction and glucose control improvement also appear to mediate the beneficial effects of exercise (Chapter 2 & 5).

Current UK physical activity guidelines recommend adults be active daily, and accumulate 150 minutes of moderate intensity or 75 minutes of vigorous intensity aerobic activity, or a combination of the two, spread across the week [661]. The guidelines also recommend doing some form of strength training on at least two days a week and for the first time, minimising sedentary time.

Assuming two 30 minute strength training sessions, following these recommendations would constitute a time commitment of 135-210 minutes per week. Many might see this as a considerable time commitment given that lack of time is a commonly reported barrier to exercise [648]. But there is also a more fundamental problem that lies in the definition of vigorous activity in these guidelines, as this intensity is framed in terms of time taken to achieve a desired energy expenditure, which Chapter 3 and 5 of this thesis show is not necessary to achieve desirable changes in cardiorespiratory fitness or markers of cardiometabolic risk. To date, there is little or no mention of a high-intensity intermittent approach to exercise, probably due to a lack of published research in this area during guideline development, and a perception that this very vigorous class of activity would not be attractive to many in the population [662].

The results presented in Chapter 5, and the review in Chapter 3, support the benefits of a high-intensity intermittent approach to exercise, where intensity is relative to the individual not absolute, and therefore feasible for most healthy and clinical populations with appropriate modifications and safety screening (Section 3.3.1). The exercise programme presented in Chapter 5 involved 90-120 minutes per week of structured exercise and resulted in marked reductions in liver fat, modest improvements in body composition despite study imposed maintenance of total body weight, and improvements in glucose metabolism. The observed changes in glucose control and body composition were in line with previous reports in broadly similar populations (*Chapter 3*). The relatively short duration should appeal to those citing time as a barrier to participate in exercise. The simplicity of the programme, and the novel use of pre-recorded auditory instructions, should appeal to clinicians without access to well developed exercise referral programmes or teams with clinical exercise prescription expertise. The caveat being that patients should always be risk assessed before starting an exercise programme.

In summary, the work in this thesis is particularly relevant to populations with NAFLD, who constitute a particularly at risk group for type 2 diabetes [282], cardiovascular disease [288], and some cancers [204,297]. The systematic review of lifestyle therapies in Chapter 2 and the study of high-intensity intermittent training in NAFLD patients in Chapter 5, demonstrate the efficacy of exercise in

reducing liver fat in those with NAFLD independent of weight reduction, albeit not necessarily independent of body composition change. Chapters 3 and 5 highlights the potential for high-intensity intermittent training to improve body body composition, and lower multiple markers of cardiometabolic risk. These findings come at a crucial time as lifestyle intervention remains the only recommended treatment for NAFLD [13,14]. Yet to date, there is no published work to suggest this is being effectively translated into clinical care. The minimally supervised high-intensity intermittent approach to exercise outlined in Chapter 5 offers one time efficient and effective way to reduce liver fat in NAFLD patients, and the materials developed for this thesis (see *Appendices 1 & 9)* provide a detailed template for clinical exercise prescription.

7.1 Future Directions

Although the benefits of various forms of exercise are well supported in terms of positive changes to many disease risk factors, several questions remain unanswered:

- What is the dose response, and more fundamentally;
- To what extent do exercise and physical activity of different types and intensities differ with respect to:
    - physiological effects;
    - acceptability among different groups;
- How do different types and intensities of physical activity interact and to what extent are they interchangeable/distinct in terms of benefits; and
- What characterisers good and what poor responders to different exercise approaches and how to identify them early so appropriate physical activity interventions can be prescribed;
- What is the optimal way to integrate physical activity / exercise and nutrition to achieve a sustained improvements in metabolic control; and
- What are the best markers to assess the effect of exercise on general health.

However, the prevalence of inactivity and poor diets [663] suggests the main challenge lies in finding effective ways to promote broad population adherence to existing physical activity and nutrition guidelines and, where needed, adherence to patient specific physical activity prescriptions.

7.2 Conclusion

The findings of this thesis are that a broad range of physical activity, exercise, and dietary approaches – one of them being high intensity intermittent training – can be prophylactic or therapeutic with respect not only to NAFLD but related non-communicable diseases. Physical activity in particular offers the possibility of improved health even in the obese and/or elderly. The benefits are largely independent of weight change, which tends to stay relatively stable, and more commonly relate to improvements in body composition, glucose control, and cardiorespiratory fitness. Future successes in translation of these findings into clinical care rest on fitting both the lifestyle prescription and means of delivery to the individual patient. In the interim, the findings in this thesis strongly suggests that the focus of clinical care teams and policy makers should be to promote attitudes and environments that encourage patients with metabolic disease to stand up and move, both physically and metaphorically, toward a future of lifelong health through physical activity.

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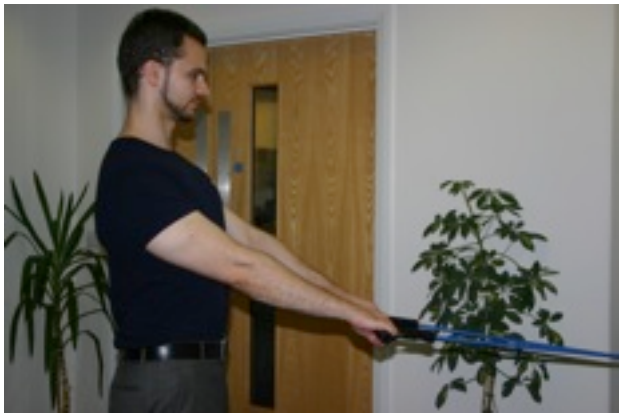
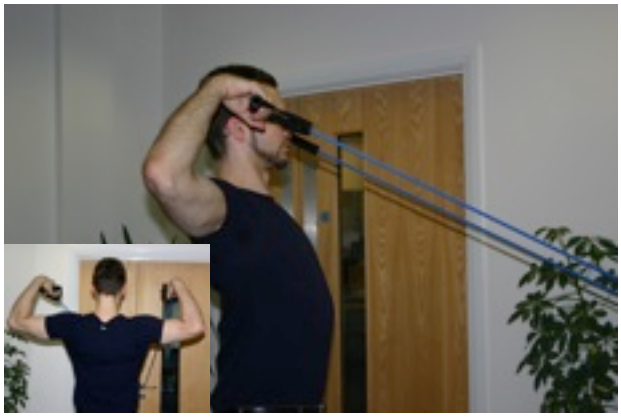
Appendix 1

Exercise Picture Guide

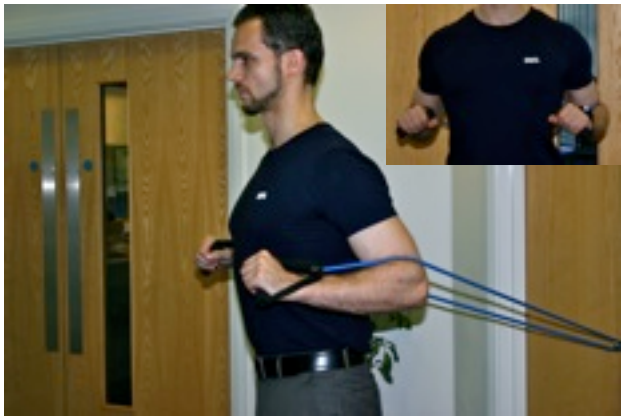
Face Pull - Start



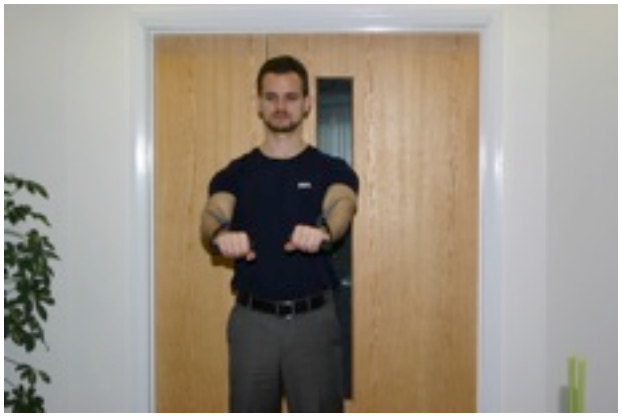
Face Pull - Finish



Chest Press - Start



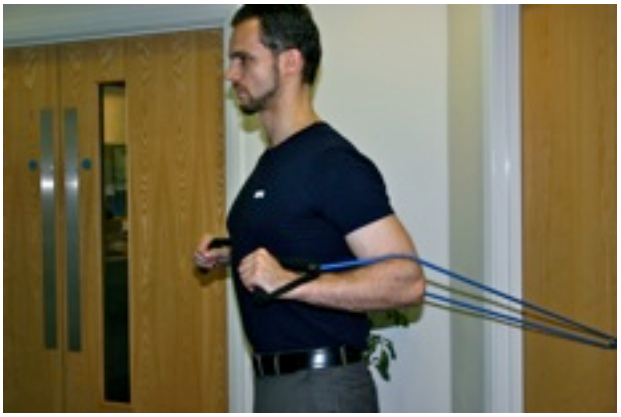
Chest Press - Finish



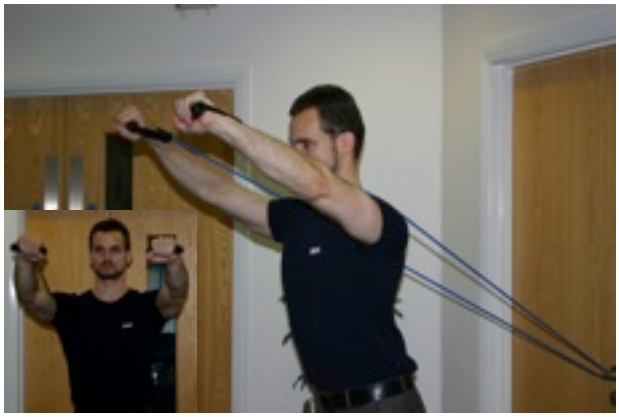
Row - Start

Row - Finish

High Press - Start



High Press - Finish



Rate of Perceived Exertion

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

Progression for warmup

Goal for intervals

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Newcastle University

REC reference number: 09/H0904/55
Committee: Sunderland Research ethics committee

Dr M I Trenell PhD, MSc, BSc (hon)
Diabetes UK RD Lawrence Fellow
Newcastle Magnetic Resonance Centre
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Patient Information Sheet

Physiological effect of age on metabolism (muscle, cardiac and abdominal) probed by MR methods, stratified for age and habitual physical activity.

You are invited to participate in this medical research project. Please take time to read the following information carefully. It explains why the research is being done and what it involves. If you have any questions about the information, you are very welcome to ask for further explanation. Thank you for reading this.

- Part 1 tells you about the purpose of this study and what will happen during the study.
- Part 2 gives more detailed information about the conduct of the study.

Discuss with others if you wish and take time to decide regarding your participation.

Part 1

What is the purpose of the research project?

Recent information has suggested that exercise may contribute to changes in metabolism for patients in different age groups. We aim to compare the effect that exercise has on metabolism and overall well being in patients in two different activity groups. We will also look at different ages of pages within these two groups.

Why have I been chosen?

You have been chosen because you are aged 20- 30 years, 40-50 years or above 65 years and do not have type 2 diabetes. The project will involve up to 72 people.

Do I have to take part?

Your participation is purely voluntary. If you decide to take part, you are still free to withdraw at any time without giving reasons and affecting your medical care. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

What will the research project involve?
You will be asked to attend the MRC Muscle Performance and Training Campus for Ageing and Vitality at Newcastle General Hospital or at the Royal Victoria Infirmary to be assessed. The study will take place over 12 weeks. Participants will attend the study centre for 4 visits in total.

Visit 1 (You should arrive for this visit having fasted overnight – 12 hours)
You will be asked to sign a consent form saying that you would like to take part in this research study. You will be asked to sit on a stationary exercise bicycle. During this test you will cycle at the same pace but how hard you are cycling will increase every minute. You will keep on cycling until you decide to stop or until pedalling becomes difficult. Whilst you are cycling you will be asked to wear a mouthpiece and a heart rate monitor (ECG). The exercise test will last between 10 - 15 minutes. At the end of the test you will feel tried but will recovery very quickly.

Total duration of visit 2 hours

Visit 2 (You should arrive for this visit having fasted overnight – 12 hours)
As you will have fasted overnight, we will take a blood sample so as to obtain fasting measures of your liver. We will also be measuring your glucose levels. For this, we will take a reading of your glucose when you have been fasted overnight and then ask you to drink 75g of glucose (410ml Lucozade). We will then take a further reading of your glucose after two hours (*This is to confirm that you do not have type 2 diabetes*). During this time we will give you something to eat and also ask you to complete two questionnaires that will give us a better understanding of your physical activity levels. At the end of this visit, we will provide you with an exercise activity monitor which you will be asked to wear on your arm for one week (if you haven't done this already). This will measure the amount of steps that you take throughout the day and will allow us to assign you to one of the two study groups in your age category (less than 7500 steps will be classed as 'sedentary', more than 12500 steps will be 'active').

Total duration of visit 2.5 hours

Visit 3 (You should arrive for this visit having fasted overnight -12 hours)
As we would like to take measurements of your liver and abdomen fat levels, we would ask you to attend this visit having fasted overnight. The samples will be collected via a blood sample form your arm. We also need to collect levels of glycogen (your bodies energy) for this we will ask you to lie flat on a bed for 10 minutes and measure your levels, repeating this measurement whilst asking you to raise your leg to two different points and at two different time points. We will also ask you (with the help of a qualified technician) to complete an assessment prior to undergoing a Magnetic Resonance Image scan (MRI) this is a scan which does not involve any radiation, but we need to make sure that there is no potential risk to you having this scan.
After the scanning we will measure your body composition to find how much muscle and fat you have in your body. In order to do this you will be asked to sit quietly for 2-3 minutes inside an egg shaped machine (BODPOD) wearing your underwear or your swimming suit.

Total duration of visit 2 hours

Visit 4
You will be scanned using an MRI scanner to look at your heart function and an MRS (Magnetic Resonance Spectrosocopy) which will look at your metabolic rate.

Total duration of visit 1.5 hours

Expenses and payments

Any travel / parking costs for helping with this research will be refunded.

What do I have to do?

You will continue with your usual treatment(s) during the project. You will be asked not to drink alcohol or exercise the day before the test days. Arrangements will be made for you to attend the research centres if necessary. Most of the assessments will be in the morning before breakfast – when asked; you should not eat before you come to the centre. *Food will be provided for you after the examination*.

What are the side effects of treatment received when taking part?

This study involves exercise so there are *no side effects* (such as may occur with drugs).

Are there any other possible disadvantages of taking part?

Giving up time to participate has to be considered.

What are the possible benefits of taking part?

Being more physically active may be beneficial to you and if sustained after the study, may help in preventing other complications such as heart disease and diabetes. You will be closely monitored and will be shown how to exercise correctly and help you become more physically fit.

What happens at the end of the research project?

At the end of the project, we shall be able to inform you of how the exercise affected you.

What if there is a problem?

If you have any concern or complaint about any aspect of the study this will be dealt with immediately by Dr Trenell. Contact details are given at the end of Part 1.

Will my taking part in the project be kept confidential?

All information obtained during the course of the research project will be kept strictly confidential. Your own GP will be informed of your participation in the project.

What will happen to the results of the research study?

The results of the project will be presented in national and international meetings and will be published in one of the medical journals. You will not be identified in any report or publication. You are welcome to have a copy of the results once they are published.

Who has reviewed the study?

Sunderland Research ethics committee.

Who are the contacts for further information?

Further information can be obtained from:

Dr D Jakovljevic PhD, MSc, BSc
Senior Research Associate
Medical School, ICM 4th Floor Williams Leech Building
Newcastle University
NE2 4HH

Tel: 0191 222 8257
d.jakovljevic@newcastle.ac.uk

Dr M I Trenell PhD, MSc, BSc (hon)
Diabetes UK RD Lawrence Fellow
Newcastle Magnetic Resonance Centre
Campus for Ageing and Vitality
Newcastle University
NE3 5JB

Tel: 0191 222 8264
Michael.I.Trenell@newcastle.ac.uk

Thank you.

****

Part 2

What if relevant new information becomes available?

If new information is published during the course of a study this can sometimes change how the research should go forward.

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time. Measurements already made would still be used if you were to agree to this.

What if there is a problem?

a) Complaints
If you have any concern or complaint about any aspect of this study you should contact Dr Trenell by phone on 0191 222 8264, or write to him at the address at the end of Part 1 of this document. If you remain unhappy you can contact Dr Lesley Hall, Research Governace Manager,Newcastle upon Tyne Hospitals NHS Foundation Trust, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP; Tel 0191 233 6161
b) Harm
In the unlikely event that something does go wrong and you suffer in any way the arrangements are as follows. If negligence of staff led to harm, then this would be covered by the Newcastle upon Tyne Hospitals Trust clinical negligence scheme. You may have to meet legal costs. If any harm was non-negligent then the hospital trust may consider a discretionary payment.

Will my taking part in the project be kept confidential?

All information obtained during the course of the research project will be kept strictly confidential. This will be achieved by storing information in password-protected computer files, and appointment information in locked filing systems within the Magnetic Resonance Centre. No individually identifiable information will be stored outside these Centres.

Analysis of the detailed results of the research: at this stage no personal information is part of the dataset. Results will be sent to participants, presented at scientific meetings and published in scientific journals without personal identification of any volunteer although thanks to the volunteers will be recorded.

Your own General Practitioner will be informed of your help with this study, and this is normal practice. The detailed results of the research tests will not be sent to anybody outside the designated research centres.

What will happen to blood samples?

The samples will be tested for fasting blood will be analysed for: full blood count, urea and electrolytes, liver blood tests, coagulation screen, creatine kinase, bicarbonate, LDL, HDL, triglycerides, cholesterol, glucose, insulin and c-peptide.
Samples will be stored until it is certain that the test results are accurate, and then they will be disposed of. During storage samples are identified only by a code number, not your name. No genetic or other tests will be carried out on the samples.

What will happen to results of the research?

The results will be presented at scientific meetings for discussion by other experts in this field. They will be written up in the form of a scientific paper and this will be intended to be published in a suitable scientific journal. As soon as the results are fully analysed after the end of the entire study you will receive a letter describing what we have found, and what implications it has for people as we get older.

Who is organising and funding the research?

This project is funded from a project grant from the Newcastle upon Tyne Biomedical Research Centre. The design and organisation of the study is the responsibility of Dr Trenell.

There is no payment to any of the researchers involved in this study. They are employed by Newcastle University to work in the NHS, to teach and to research and have no financial link with the study.

Who has reviewed the study?

Ethical review of the study has been conducted by the Newcastle and North Tyneside Research Ethic Committee No. 1.

Design of this information sheet

This document is written in accordance with the requirements of the European Clinical Trials Directive 2001/20/EC, the ICH Good Clinical Practice guidelines and the UK Medicines for Human Use (Clinical Trials) Regulation 2004.

Newcastle University

MRC Muscle Performance and Training
Campus for Ageing and
Vitality
Westgate Road
Newcastle upon Tyne
NE4 5PL
T: +44 (0)191 222 8264

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Patient Identification number for this trial:

CONSENT FORM

Title of Project: Physiological effect of age on metabolism (muscle, cardiac and abdominal) probed by MR methods, stratified for age and habitual physical activity

Name of researcher: Dr M Trenell
Dr D Jakovljevic

Please initial box

1. I confirm that I have read and understand the information sheet dated September 2009 (version 1.0) 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 participant	Date	Signature
Name of person taking consent (if different from participant)	Date	Signature
Researcher	Date	Signature

☐ 1 for participant; 1 for researcher; 1 to be kept with hospital notes

Visit 1

Physical Activity Readiness Questionnaire

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	Do you have any breathing problems?	YES	NO
8	Do you have any problems with your liver, thyroid, kidneys or have diabetes?	YES	NO
7	Are you currently taking any *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?		
10	How many times a week do you do exercise: _____		
11	Is there any other reason why you should not participate in physical activity? If so, what? _____	YES	NO

Signed by (staff): _____ Print: _____

Date: _____

Appendix 2 **Risk Stratification Sheet**

Name: _____

DOB: _____ **Age:** _____

Study ID: _____

General Practitioner: _____

GP Address: _____

_____

Height _____

Weight _____

Waist _____

Hip circ. _____

Blood Pressure _____

Smoker **YES** **NO** **or** **given up <6month**

Blood Taken: **YES** **NO**

Comment: _____
_____
_____
_____

To be completed by investigator:

Risk Stratification (circle): Low Moderate High

Action Taken: Commence exercise (Low Risk) YES / NO

Referred for Exercise ECG (moderate and high risk) YES / NO

Competed by _____ **Date** _____

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 Information Sheet

Effect of exercise on non-alcoholic fatty liver

You are invited to participate in this medical research project. Please take time to read the following information carefully. It explains why the research is being done and what it involves. If you have any questions about the information, you are very welcome to ask for further explanation. Thank you for reading this.

- Part 1 tells you about the purpose of this study and what will happen during the study.
- Part 2 gives more detailed information about the conduct of the study.

Discuss with others if you wish and take time to decide regarding your participation.

Part 1

What is the purpose of the research project?

Recent information suggests that exercise may help people with non-alcoholic fatty liver disease reduce the amount of fat in the liver by: 1) increasing the ability of the body to burn fat, and 2) increasing the sensitivity of the body to food.

We aim to show the effect of exercise on the levels of liver fat, sensitivity of the body to food, and heart function. We will measure liver fat, and how well your heart works using a magnetic resonance (MRI) scanner. This research DOES NOT require any biopsies.

Understanding the relationship between exercise and liver fat is important in gaining acceptance of exercise in the management of fat in the liver and avoiding excess weight gain.

Why have I been chosen?

You have been chosen because you have too much fat in your liver (called non-alcoholic fatty liver disease) and currently do not do any regular exercise. People with too much fat in their liver will be asked to help with the research project, as they could benefit from the results of this research. The project will involve up to 28 people.

Do I have to take part?

Your participation is purely voluntary. If you decide to take part, you are still free to withdraw at any time without giving reasons and without any bad feelings. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

What will the research project involve?

You will be asked to attend the Newcastle Magnetic Resonance Centre at the Newcastle General Hospital, or the Royal Victoria Infirmary on 5-9 occasions over 3 months to have your metabolism and liver fat checked. The number of visits will depend on whether you choose to do longer visits with more tests at each visit or shorter visits with the tests more spaced out. If you take part in the exercise group you will also be asked to do prescribed exercise 3 times a week for 12 weeks. What time of day you do these will only be limited by the opening hours of the gym you join.

Visit 1: Screening Visit

This visit will determine if you are suitable for this study and it is suitable for you. You will be asked to sign the consent form saying that you would like to take part in this research study, and then complete two screening questionnaires. You will then do a cycling test. During this test you will cycle at the same pace but how hard you are cycling will steadily increase. You will keep on cycling until you decide to stop or until pedalling becomes difficult. Whilst you are cycling you will be asked to wear a breathing mask and a heart monitor. The exercise test will last 10 - 15 minutes. At the end of the test you will feel tired but will recover very quickly. **Total visit time: 1 hour**

Magnetic Resonance Scans

The level of fat in your liver and how well your heart works will be measured using magnetic resonance imaging (MRI). During this test you will be asked to lie on your back for about 60 minutes and then on your front for about 45 minutes in the MRI machine. This DOES NOT involve a biopsy, is completely painless and does not have any dangers associated with the examination. **Total procedure time: 2 hours**

Oral Glucose Tolerance Test

After an overnight fast, you will be asked to drink a large sugary drink (350 ml Lucozade). A cannula (small plastic tube) will be placed in your arm so that blood samples can be collected at regular intervals for 2 hours after you drink the sugary drink. We usually do this after one of the scans. **Total procedure time: 2.5 hours**

Body Fat Measurement

The amount of fat and muscle in your body will be measured whilst you sit quietly on a chair for 5 minutes. We usually do this before one of the longer procedures. **Total procedure time: 10 minutes**

Resting Metabolism and Exercise Test

After an overnight fast, you will be asked to lie quietly on a bed whilst your resting metabolism is measured. Then you will be asked to cycle for 60 minutes at an easy pace. You can watch a DVD or listen to music whilst you cycle. A cannula (small plastic tube) will be placed in your arm so that blood samples can be collected at regular intervals during exercise. You will also be asked to wear a breathing mask for some of the time, and a heart rate monitor. **Total visit time: 2.5 hours**

Except for Visit 1, all the tests will be done once at the start of the study and once at the end of the study

You will be assigned to one of two groups. The *first group* will do 3 exercise sessions per week over 12 weeks. Each exercise session will involve you using a stationary cycle and resistance band, and will last about 40 minutes. These sessions will be held at a local gym that you choose, your gym membership will be paid for during the study. A member of the research team will come with you on your first session to familiarise you with doing the exercises safely. It is important that your weight does not change over this time. You will be given food supplements (protein milkshake) if your weight goes down by more than 1%. The *second group* will not do the exercise sessions or be required to undertake any exercise over the 12 weeks. You will be asked to wear a physical acitivity monitor for 7 days before and for 7 days toward the end of the12 week study period.

If you are placed in the group which does not attend any exercise sessions, at the end of the study you will be given the opportunity to receive an exercise prescription after finishing the study, though this is not compulsory. No further tests will be required.

Expenses and payments

Any travel / parking costs for helping with this research will be refunded.

What do I have to do?

You will continue with your usual treatment(s) during the project. It will be important that you attend at least 32 of the possible 36 exercise sessions. You will be asked not to drink alcohol or exercise the day before your visits. There is parking outside the Magnetic Resonance Centre, or if you wish, taxi transport will be arranged. Each of the metabolic assessments will be in the morning before breakfast – you should not eat before you come for your visit. Food will be provided for you after the examination.

What are the side effects of treatment received when taking part?

This study involves exercise so there are no side effects (such as may occur with drugs).

Are there any other possible disadvantages of taking part?

Giving up time to participate has to be considered.

What are the possible benefits of taking part?

Being more physically active may be beneficial to the level of fat in your liver and if sustained after the study, may help in preventing other complications such as heart disease and diabetes. You will have supervised exercise sessions (like a personal trainer) which will teach you about your body, show you how to exercise correctly and help you become more physically fit.

What happens at the end of the research project?

At the end of the project, we shall be able to inform you of how the exercise affected liver fat level and metabolism.

What if there is a problem?

If you have any concern or complaint about any aspect of the study this will be dealt with immediately by Dr Trenell. Contact details are given at the end of Part 1.

Will my taking part in the project be kept confidential?

All information obtained during the course of the research project will be kept strictly confidential. Your own GP and liver doctor will be informed of your participation in the project.

What will happen to the results of the research study?

The results of the project will be presented at national and international medical meetings and will be published in one or more appropriate scientific journals. You will not be identified in any report or publication. You will be welcome to have a copy of the results once they are published.

Who has reviewed the study?

Ethical review of the study has been conducted by the Newcastle and North Tyneside Research Ethic Committee No. 1.

Who are the contacts for further information?

Further information can be obtained from:

Christian Thoma or Kate Hallsworth
Institute of Cellular Medicine
William Leech Building
Framlington Place
Newcastle University
NE2 4HH
Tel 0191 222 8264
Email: christian.thoma@ ncl.ac.uk or kate.hallsworth@ncl.ac.uk

Thank you.

****

Part 2

What if relevant new information becomes available?

If new information is published during the course of a study this can sometimes change how the research should go forward. However, for this study it is most unlikely that this would occur. The study design is unique, and there are very few research groups worldwide able to carry out magnetic resonance-based research of this kind.

What will happen if I don't want to carry on with the study?

You would be able to withdraw from the study at any time. Measurements already made would still be used if you were to agree to this.

What if there is a problem?

a) Complaints
If you have any concern or complaint about any aspect of this study you should contact Dr M Trenell by phone on 0191 248 1150, or write to him at the address at the end of Part 1 of this document. If you remain unhappy you can contact the Research Manager of the Newcastle upon Tyne Hospitals NHS Trust (Dr C Mackerness, Clinical Research Centre, RVI, Queen Victoria Road, Newcastle upon Tyne NE1 4LP; Tel 0191 282 5959).

b) Harm
In the unlikely event that something does go wrong and you suffer in any way the arrangements are as follows. If negligence of staff led to harm, then this would be covered by the Newcastle upon Tyne Hospitals Trust clinical negligence scheme. You may have to meet legal costs. If any harm was non-negligent then the hospital trust may consider a discretionary payment.

Will my taking part in the project be kept confidential?

All information obtained during the course of the research project will be kept strictly confidential. This will be achieved by storing information in password-protected computer files, and appointment information in locked filing systems within the Clinical Research Facility, Royal Victoria Infirmary. No individually identifiable information will be stored outside the hospital.

Analysis of the detailed results of the research will be done by Dr Trenell, Dr Hollingsworth, Professor Taylor and Professor Day. At this stage no personal information is part of the dataset. Results will be sent to participants, presented at scientific meetings and published in scientific journals without personal identification of any volunteer although thanks to the volunteers will be recorded.

Your own General Practitioner will be informed of your help with this study; this is normal practice. Similarly, if you attend a specialised clinic for care, your consultant will be informed. The detailed results of the research tests will not be sent to anybody outside the research team.

What will happen to blood samples?

The samples will be tested for liver function, insulin, sugar, fat, and other food derived substances. Samples will be stored until it is certain that the test results are accurate, and then they will be disposed of. During storage samples are identified only by a code number, not your name. No genetic or other tests will be carried out on the samples.

What will happen to results of the research?

The results will be presented at scientific meetings for discussion by other experts in this field. They will be written up in the form of a scientific paper for publication in a suitable journal. As soon as the results are fully analysed after the end of the entire study, you will receive a letter describing what we have found, and what implications it has for people with non-alcoholic fatty liver disease.

Who is organising and funding the research?

This project is funded by a project grant from the Newcastle Hospitals Trust Joint Research Committee. The design and organisation of the study is the responsibility of Dr Trenell, Professor Taylor and Professor Day who are internationally recognised as experts in this field.

There is no payment to any of the researchers involved in this study. They are employed by Newcastle University to work in the NHS, to teach and to research and have no financial link with the study.

Who has reviewed the study?

Ethical review of the study has been conducted by the Newcastle and North Tyneside Research Ethic Committee No. 1.

Design of this information sheet

This document is written in accordance with the requirements of the European Clinical Trials Directive 2001/20/EC, the ICH Good Clinical Practice guidelines and the UK Medicines for Human Use (Clinical Trials) Regulation 2004.

Institute of Cellular Medicine
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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

1. 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. ☐
2. I understand that my participant is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to my GP being informed of my participation in the study ☐
4. I agree to take part in the above study. ☐
5. I 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

09 April
Ver 1-1 | 09

Risk definition and Standard Operating Procedures for Exercise Testing;

MRC Muscle Performance & Exercise Training Laboratory

Dr Mike Trenell
Institute of Cellular Medicine
Newcastle University

Version 1.1

Table of contents

Executive Summary

This document contains two sections. The first section will define the risks associated with exercise and exercise testing of people from the general population. The second section will describe the pre-exercise screening procedures and operating procedures which will be used at the MRC Muscle Performance and Training Laboratory. The document will also outline the evidence base underlying the protocols for exercise testing and pre-exercise screening.

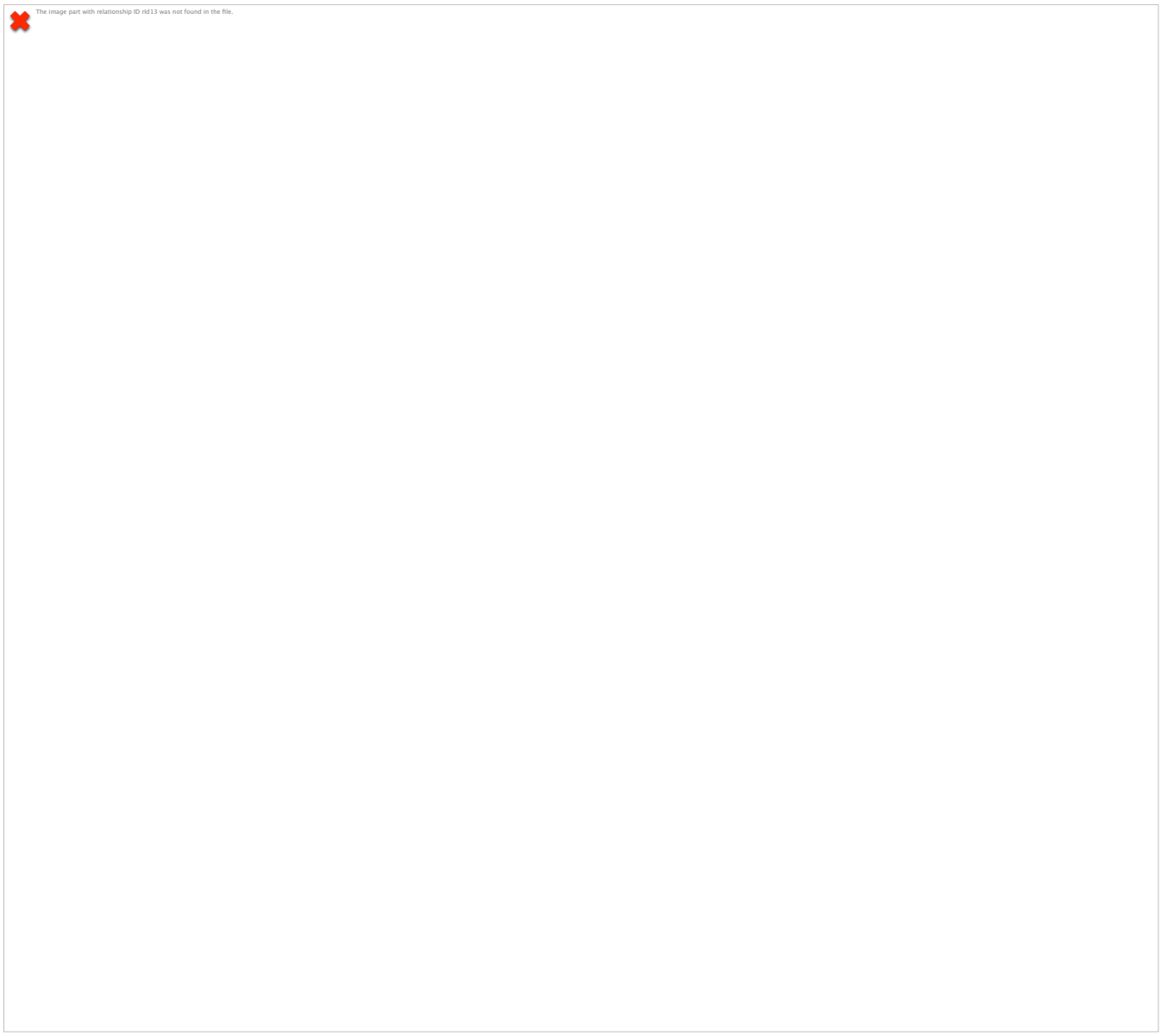
Habitual physical activity and exercise reduces all cause mortality. Nevertheless, vigorous exercise (>75% age predicted maximal heart rate) carries risks. In a mixed population or predominantly older people, the risk of a cardiac arrest during maximal progressive exercise testing is approximately 3 events per 10,000 tests.

It is important to remember that exercise only provokes cardiovascular events in people with pre-existing heart disease, whether diagnosed or occult. Exercise, whether maximal or sub-maximal, does not provoke cardiovascular events in individuals with normal cardiovascular systems. The MRC Muscle Performance and Exercise Training Laboratory will only study subjects with moderate or high risk of having ischemic heart disease after exclusion by formal exercise testing. Screening for undiagnosed ischemic heart disease will be carried out in the Clinical Research Facility where there is on-site cover by the acute hospital resuscitation team. A flow diagram describing this is shown in Figure 1.

It is projected that the MRC Muscle Performance and Exercise Training Laboratory will investigate approximately 100 people and undertake 250 exercise tests per year (100 of which are screening tests). Assuming that the risks are approximately one quarter that of general population exercise risks, as all people will be screened for ischemic heart disease, this produces a conservative estimated cardiac arrest rate of one every 50 years.

This document outlines the level of staff qualifications, emergency procedures and screening procedures which will be used for exercise testing at the MRC Muscle Performance and Exercise Training Laboratory to appropriately minimise and manage the risks to research volunteers associated with exercise testing and prescription.

Figure 1 Schematic representation of the study participant screening pathway and related Standard Operating Procedure (SOP) defining these.



SECTION 1 – RISK DEFINITION

Exercise related cardiac events in adults

The risks of exercise in older adults (over 50 yr of age) are considerably higher than in younger subjects because of the increased prevalence of atherosclerotic cardiovascular disease. The most widely cited studies, performed in Rhode Island (22) and Seattle (20), estimate an incidence of sudden cardiac death during vigorous, unsupervised physical exertion in healthy adults as one death per year for every 15,000 to 18,000 individuals. This is a low incidence of cardiovascular events, but both studies demonstrated that the rate of sudden cardiac death during or immediately after vigorous exertion was higher than that for more leisurely activities. Exercise is associated with an increased incidence of acute nonfatal myocardial infarction (12, 18). Both the incidence of exertion-related sudden cardiac death (20), and acute myocardial infarction (12, 18) are higher in individuals who exercise infrequently.

The mechanism of sudden cardiac death and acute myocardial infarction in previously asymptomatic adults is thought to be acute coronary plaque rupture leading to coronary thrombosis. Such atherosclerotic plaque disruption with acute thrombotic occlusion has been documented by angiography in individuals with exercise induced cardiac events (4, 5, 13).

Risks of cardiac events during exercise testing

The risk of exercise varies with the prevalence of underlying coronary artery disease in the population. Consequently, the risk of exercise stress testing also varies with the populations studied. Exercise stress testing performed in previously healthy individuals has a very low rate of cardiovascular events, whereas exercise testing in high-risk patients has a higher risk. The overall risk of exercise stress testing in a mixed population is approximately 4 cardiac events (i.e. 1 myocardial infarction and 3 cardiac arrests) per 10,000 tests (see Table 1). These results include exercise testing supervised by non-physicians (15).

Prevention of exercise-related cardiac events

The development and evaluation of strategies to reduce the risk of vigorous exercise is complicated by the low incidence of events. Interventions cannot be proposed and tested because an enormous number of subjects would have to participate in order for this to achieve sufficient statistical power.

Consequently, the recommendations for strategies to reduce risk cardiac events are based primarily on expert opinion and consensus. Furthermore, the paucity of exercise related cardiac events make it difficult to quantify the benefits of any routine screening procedures. Some experts recommend extensive pre-participation screening prior to sports participation including electrocardiography (16) and echocardiography (6). However, this approach is controversial and not broadly supported (1).

The ACSM (1) and AHA (11) recommends exercise stress testing prior to initiating exercise for "moderate" or "high risk" people (these groups are defined in Table 3, Page 6), including men over 45 and women over 55 years of age, individuals with more than one cardiovascular disease risk factor, and those with known cardiovascular disease.

Procedures for reducing the risks of vigorous exercise during exercise training, exercise stress testing, and cardiac rehabilitation have not been tested rigorously (1).

Table 1 Cardiac complications during exercise testing in suspected ischemic heart disease patients. Total events for test and following days expressed per 10,000 tests.

Reference	Year	Site	No. Tests	MI	VF	Death	Hospitalisation	Comments
Rochmis (19)	1971	73 U.S.	170,000	NA	NA	1	3	34% of tests wee symptom limited; 50% of deaths in 8hr; 50% over the next 4 days
Irving (14)	1977	15 Seattle facilities	10,70	NA	4.67	0	NR	
McHenry (17)	1977	Hospital	12,000	0	0	0	0	
Atterhög (3)	1979	20 Swedish Centres	50,000	0.8	0.8	6.4	5.2	
Stuart (21)	1980	1,375 U.S. Centres	50,000	3.6	4.8	0.5	NR	"VF" includes other dysrthythmias requiring treatment
Gibbons (9)	1989	Cooper Clinic	518,448	0.6	0.3	0	NR	Only 4% of men and 2% of women had CAD
Knight (15)	1995	Geisinger Cardiology Service	28,133	1.4	1.8	0	NR	25% were in-patient tests supervised by non-MD's
Average				**1.28**	**2.06**	**1.13**	**2.73**	

Abbreviations: MI, myocardial infarction; VF, ventricular fibrillation; CAD, coronary artery disease; MD, medical doctor; NA, not applicable; NR, not reported.

Table 2 Number of patients for one cardiac event in cardiac rehabilitation programs*

Reference	Cardiac Arrests	MI	Fatalities	MI and Arrest
Van Camp (23)	111,996	293,990	783,972	81,101
Dgenio (7)	120,000	-	160,000	120,000
Vongvanich (24)	89,501	268,503	268,503	67,126
Franklin (8)	146,127	97,418	292,254	58,451
Average	**116,906**	**219,970**	**732,364**	**81,669**

Adapted from Franklin *et al.* Safety of medically supervised outpatient cardiac rehabilitation exercise therapy: a 16 year follow up. Chest 1998; 114:902-906 (8)

* Cardiac rehabilitation programs are in general relatively high intensity exercise sessions (>70% maximum heart rate).

SECTION 2 – RISK MANAGEMENT

As shown in Figure 1 (page 2), there are three key levels of risk management at the MRC Muscle Performance and Exercise Testing Laboratory. These follow the guidelines on exercise testing and prescription identified by the American College of Sports Medicine and the American Heart Association. We will now outline what these stages are and also the Standard Operating Procedure describing how they should be done, where they should be done and by whom.

The first stage is risk stratification, classifying the participant as Low, Moderate, or High risk of having undiagnosed heart disease. These are described in the standard Operating Procedure MRC PETL 01.

The second stage of risk management is screening Moderate and High risk of undiagnosed heart disease individuals for undiagnosed heart disease and an adverse reaction to exercise. These are described in the standard Operating Procedure MRC PETL 02.

The final stage is managing any adverse events whilst undergoing exercise training. These are described in the standard Operating Procedure MRC PETL 03.

Standard operating Procedure: MRC PETL 01

Risk Stratification

MRC Muscle Performance and Exercise Training Laboratory
Standard Operating Procedure

SOP number:	MRC-PETL 01
SOP full title:	Risk stratification
SOP category:	
SOP effective:	Review date:

SOP author signature: Date:

Dr Michael Trenell
Scientific Director, MRC Muscle Performance and Training Laboratory

SOP Reviewer signature: Date:

<name>, <title>

SOP approval signature: Date:

<name>, <title>

Background

Standard Operating Procedures (SOPs) are designed to ensure that clinical research, and its supporting activities, is conducted to the principles of Good Clinical Practice (GCP). GCP is an international ethical and scientific quality standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of trials that involve the participation of human subjects. Compliance with GCP provides assurance that the data and reported results are credible and accurate, and that the rights, wellbeing and safety of participants are protected.

All participants in research projects being undertaken at the MRC Muscle Performance and Exercise Training laboratory must be protected from harm, undue risk and experience a friendly environment.

Purpose

To identify the risk stratification process prior to commencing exercise.

Scope

This SOP applies to all exercise studies undertaken by participants at MRC Muscle Performance and Exercise Training Laboratory. It will define the procedures, criteria and level of professional certification for risk stratification.

Procedure

1. Risk stratification

The risk of the patient having undiagnosed coronary artery disease, and as a consequence an increased risk of an adverse cardiac response to exercise, will be
determined by using the American College of Sports Medicine (1) pre-participation screening algorithm (Table 1). The first stage of this, defined in the Standard Operating Procedure, is Risk Stratification.

During a screening consultation, participants will complete the Physical Activity Readiness Questionnaire (Appendix 1) to provide details about their medical history Body weight, height, waist and hip circumference and blood pressure will be assessed. Where there is insufficient information to determine Risk Stratification, blood will be collected for evaluation of a lipid profile and fasting glucose.

Using the screening algorithm in Table 2 and 3 (defined by the American College of Sports Medicine (1)), participants will then be stratified into Low, Moderate, and High Risk of having coronary artery disease and as a consequence an adverse response to exercise.

Following the ACSM pre-participation screening algorithm (Table 1), participants with Moderate or High risk of undiagnosed coronary artery disease will undergo further cardiac examinations (detailed in the SOP MRC PETL 02) for assessment of cardiac function and adverse response to exercise before commencing further exercise. Participants with Low risk of undiagnosed coronary artery disease will not require any additional screening before commencing further exercise.

2. Pathway for dealing with undiagnosed risk factors

Any undiagnosed complications which arise from the Risk Stratification, such as hypertension, dyslipidemia, impaired glucose control, or abnormal liver function teats will be notified to the participants general practitioner in writing by the principle investigator.

A decision about the participant continuing in the research study will be based upon the more detailed evaluation outlined in the standard operating procedure MRC PETL 02.

3. Certification for tasks.

Risk stratification will be undertaken by a physician, nurse, physiotherapist, or clinical exercise physiologist following this SOP guideline.

4. Reporting

The principle investigator will be responsible for administering and retaining copies of Risk Stratification documentation (Physical Activity Readiness Questionnaire, International Physical Activity Questionnaire, and Risk Identification).

The principle investigator is responsible for referring the study participant to appropriate clinical support (general physician) following any abnormal observations during the study.

References

1 American College of Sports Medicine. ACSM's guidelines for Exercise Testing and Prescription. Ed: Whaley MH, Lippincott, Williams & Wilkins, Philadelphia, 7th Edition, 2006.

Table 1 Pre-participation screening algorithm

Adapted from the ACSM pre-participation screening algorithm (1).

	Screening required prior to professional-guided exercise testing/prescription		
Level 1	**1** – Determine risk category from Table 3 using Physical Activity Readiness Questionnaire **2** – Determine need for medical clearance prior to testing and/or participation and obtain if recommended **3** – Proceed to level 2 and follow recommendations		
Level 2	Low Risk	Moderate Risk	High risk
	• Perform informed consent for testing • Complete appropriate assessment procedure • No requirement for pre-exercise screening for cardiovascular disease • Medical history	• Perform informed consent for testing • Intensity of pre-exercise test assessment should increase as a function of risk category.	
Level 3	• Further medical examination and exercise not necessary prior to initiation of exercise	• Medical examination and exercise testing * recommended prior to initiation of vigorous exercise training • Qualified† supervision recommended for *maximal* exercise testing	• Medical examination and exercise testing* recommended prior to initiation of vigorous exercise training • Qualified† supervision recommended for *maximal* and *sub-maximal* exercise testing.

† When qualified supervision is recommended, there should be at least two people with up to date ICLS qualifications present at all times and who are familiar with the local emergency procedures.

NB: Level 2 and 3 dealt with in SOP MRC-PETL 02

Table 2 Risk Stratification Categories *adapted from the American College of Sports Medicine guidelines (1).*

Low Risk	Men <45years of age and women <55 years of age who are asymptomatic and meet no more than one risk factor threshold from Table 4.
Moderate Risk	Men >45 years and women >55 years or those who meet the threshold for two or more risk factors from Table 4.
High Risk	Individuals with one or more signs of cardiovascular disease[+] and symptoms listed in Table 4 or known cardiovascular*, pulmonary[#], or metabolic disease[†].

[+] Ankle oedema, palpitations or tachycardia, intermitted claudication, known heart murmur, unusual fatigue or shortness of breath with usual activities.

* Cardiac, peripheral vascular, or cerebrovascular disease.

[#] Chronic obstructive pulmonary disease, interstitial lung disease, or cystic fibrosis.

[†] Diabetes mellitus (IDDM, NIDDM), thyroid disorders, renal or liver disease.

Table 3 Coronary artery disease risk factor thresholds for use with risk stratification.

Positive Risk Factor	Defining Criteria
Family History	Myocardial infarction, coronary revascularisation, or sudden death before 55 years of age in father or other male first-degree relative, or before 65 years of age in mother or other female first degree relative.
Cigarette Smoking	Current cigarette smoker or those who quit within the previous 6 months.
Hypertension	Systolic blood pressure >140Hg or diastolic >90 mm Hg, confirmed by measurements on at least two separate occasions, or on antihypertensive medications
Dyslipidemia	Low-density lipoprotein (LDL) cholesterol >3.4mmol.l or high density lipoprotein (HDL) cholesterol <1.03 mmol.l or on lipid lowering medication. If serum cholesterol is all that is available use >5.2mmol.l rather than >3.4mmol.l.
Impaired fasting glucose	Fasting blood glucose > 5.6mmol.l
Obesity	BMI >30 $kg.m^2$, or Waist girth >102 for men and 88 cm for women, or Waist / hip ratio > 0.95 for men and >0.86 for women
Sedentary lifestyle	Persons not participating in a regular exercise program or not meeting the minimal physical activity recommendations from the US surgeon general[†]

[†] Accumulating 30 minutes or more of moderate physical activity on most days.

Appendix 1 Modified Physical Activity Readiness Questionnaire

Physical Activity Readiness Questionnaire

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	Do you have any breathing problems?	YES	NO
8	Do you have any problems with your liver, thyroid, kidneys or have diabetes?	YES	NO
7	Are you currently taking any *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?		
10	How many times a week do you do exercise: _____		
11	Is there any other reason why you should not participate in physical activity? If so, what? _____	YES	NO

Signed by (staff): _____ Print: _____

Date: _____

Appendix 2 Risk Stratification Sheet

Name: _____

DOB: _____ **Age:** _____

Study ID: _____

General Practitioner: _____

GP Address: _____

_____

Height _____

Weight _____

Waist _____

Hip circ. _____

Blood Pressure _____

Smoker **YES** **NO** **or** **given up <6month**

Blood Taken: **YES** **NO**

Comment: _____
_____
_____
_____

To be completed by investigator:

Risk Stratification (circle): Low Moderate High

Action Taken: Commence exercise (Low Risk) YES / NO

Referred for Exercise ECG (moderate and high risk) YES / NO

Competed by _____ **Date** _____

Standard operating Procedure: MRC PETL 02

Physical examination and exercise testing

MRC Muscle Performance and Exercise Training Laboratory
Standard Operating Procedure

SOP number:	MRC PETL 02
SOP full title:	Pre-exercise training stress testing
SOP category:	
SOP effective:	Review date:

SOP author signature: Date:

Dr Michael Trenell
Scientific Director, MRC Muscle Performance and Training Laboratory

SOP Reviewer signature: Date:

<name>, <title>

SOP approval signature: Date:

<name>, <title>

Background

Standard Operating Procedures (SOPs) are designed to ensure that clinical research, and its supporting activities, is conducted to the principles of Good Clinical Practice (GCP). GCP is an international ethical and scientific quality standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of trials that involve the participation of human subjects. Compliance with GCP provides assurance that the data and reported results are credible and accurate, and that the rights, wellbeing and safety of participants are protected.

All participants in research projects being undertaken at the MRC Muscle Performance and Exercise Training laboratory must be protected from harm, undue risk and experience a friendly environment.

Purpose

To identify the process of pre-exercise evaluation of cardiac function and response to progressive exercise in people classified as Moderate or High risk of undiagnosed heart disease following screening (defined in SOP MRC PETL 01).

Scope

This SOP applies to all pre-exercise evaluation of cardiac function in Moderate and High Risk patients (defined in SOP MRC PETL 01) undertaken at the MRC Muscle Performance and Exercise Training Laboratory. The SOP will define:

1. Evaluation of Medical History
2. The procedures for evaluating cardiac function
3. The criteria for successful completion.
4. Where these should be undertaken
5. How many people are required
6. What level of certification is required to undertake these tests
7. How people will be certified to begin exercise
8. Referral of study participant following unsuccessful screening
9. Management of cardiac event

Procedures

1. *Screening*
    a. Medical history

        Participants be screened for their medical history by a physician or qualified exercise specialist* (*Appendix 1 – Evaluation of Medical History*). ** see section 5*

    b. Physical examination

        Participants be undergo a physical examination by a physician or qualified exercise specialist* (Appendix 2 – Physical Examination Sheet). ** see section 5*

2. *The procedures for undertaking an exercise stress test*

Study participants classified as being at Moderate or High Risk of having undiagnosed heart disease (defined in SOP MRC PETL 01) will undergo an exercise stress test. During this evaluation, expired gases, respiration, blood pressure and cardiac function will be monitored.

a. The procedures for undertaking an exercise stress test

The exercise stress examination will have two stages; 1) rest, and 2) exercise. Expired gases, respiration, blood pressure and cardiac function will be assessed throughout both of these periods. The exercise stress test will be terminated when any of the absolute contraindications listed in Appendix 3 occur.

3. *The criteria for successful completion.*

The resting and exercise test should be completed without the occurrence of any absolute contraindications to exercise. Absolute contraindications are listed in Appendix 3.

4. *Level of supervision*

During exercise testing, two people will be present at all times.

5. Level of certification

Medical history, physical examination and exercise evaluation must be undertaken by a physician or by an exercise specialist holding current American College of Sports Medicine Clinical Exercise Specialist or Clinical Exercise Physiologist certification. These certifications are the highest possible level of clinical certification for exercise testing and prescription.

The Knowledge, Skills and Attributes (KSA's) covered in the American College of Sports Medicine certification include: Health Appraisal and Fitness Exercise Testing, Exercise Prescription (Training) and Programming, Electrocardiography and Diagnostic Techniques, Exercise Physiology and Related Exercise Science, Pathophysiology and Risk Factors, Human Behaviour, Safety, Injury Prevention and Emergency Procedures, Nutrition and Weight Management, Patient Management and Medications, Program Administration, Quality Assurance, and Outcome Assessment, Medical and Surgical Management. See www.acsm.org/ for further details.

During exercise testing, both supervising investigators should hold at least current intermediate cardiac life support (ICLS) qualifications.

6. Where will exercise testing be undertaken

Pre-exercise screening of low risk participants requires the supervision of two ICLS trained staff and no Advanced Cardiac Life Support (ACLS) trained team. These tests will be completed at the MRC Muscle Performance and Exercise Training Laboratory, based at the Clinical ageing Research Unit at the Campus for Ageing and Vitality.

Pre-exercise screening of moderate and high-risk groups must be undertaken in the presence of an ACLS team as well as the two supervising ICLS trained individuals. Testing of moderate and high risk study participants will be undertaken at the Clinical Research Facility at the Royal Victoria Infirmary.

All exercise testing must be undertaken with automated defibrillators available for use and the supervising personnel familiar with their use.

7. How study participants will be certified to begin exercise

Upon successful completion of the screening procedures, the resting and exercise ECG will be certified by the supervising physician as not having undiagnosed heart disease that would produce an adverse reaction to exercise.

Successful completion of the screening procedures will be documented in the Physical Examination form (Appendix 2).

8. Referral of study participant following unsuccessful screening

If a study participant fails the screening process they will be referred back to their general physician. A screening report will be provided by the Principle Investigator to the general physician and the study participant advised not to undertake exercise until the general physician has cleared them to do so.

9. Management of an adverse cardiac event

All exercise screening will be undertaken at the Clinical Research Facility at the Royal Victoria Infirmary. As such, the Newcastle Hospitals Trust Cardiac Event Policy will be followed.

Exercise testing undertaken at the MRC Muscle Performance and Exercise Training Laboratory within the Clinical Ageing Research Unit will follow the local guidelines for management of an adverse cardiac event.

Table 1 Pre-participation screening algorithm. *Adapted from the ACSM pre-participation screening algorithm*.

	Screening required prior to professional-guided exercise testing/prescription		
Level 1*	**1** – Determine risk category from Table 2 using Physical Activity Readiness Questionnaire **2** – Determine need for medical clearance prior to testing and/or participation and obtain if recommended **3** – Proceed to level 2 and follow recommendations		
Level 2	Low Risk	Moderate Risk	High risk
	• Perform informed consent for testing • Complete appropriate assessment procedure • No requirement for pre-exercise screening for cardiovascular disease • Medical history	• Perform informed consent for testing • Intensity of pre-exercise test assessment should increase as a function of risk category.	
Level 3	• Further medical examination and exercise not necessary prior to initiation of exercise	• Physical examination and exercise testing recommended prior to initiation of vigorous exercise training • Qualified† supervision recommended for *maximal* exercise testing	• Physical examination and exercise testing recommended prior to initiation of vigorous exercise training • Qualified† supervision recommended for *maximal* and *sub-maximal* exercise testing.

* The procedures for Level 1 assessment are detailed in MRC PETL SOP1

Appendix 1 Evaluation of Medical History

(*on reverse of Physical Activity Readiness Questionnaire from SOP MRC PETL 01*)

Medical Diagnosis:

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

Previous Physical Examination

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

History of symptoms

Discomfort in the chest, jaw, neck, back or arms (e.g. *pressure, tingling, pain, heaviness, burning, tightness, squeezing or numbness*)	YES	NO
Light headedness , dizziness or faint?	YES	NO

Recent Illness

Hospitalisation, new medical diagnosis, surgery	YES	NO

Details _____

_____

Orthopaedic problems

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

Medication use

Medication	YES	NO

Details _____

_____

Allergies

Details _____

_____

Other habits

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

Exercise history

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

Work history

Focus on current of expected physical demands

Family history

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

Comments:

_____

_____

_____

Competed by _____ **Date** _____

Appendix 2 Physical examination (page 1 – page 2 on the next page)

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

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

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

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

Resting blood pressure, seated. ___ / ____

Auscultation of the lungs *with specific attention to uniformity of breath sounds in all areas (absence of rales and wheezes)*	**OK / Not OK** *Comment:*_____
Palpation of cardiac apical impulse *point of maximal impulse*	**OK / Not OK** *Comment:*_____
Auscultation of the heart *with specific attention to murmurs, gallops, clicks and rubs.*	**OK / Not OK** *Comment:*_____
Evaluation of the abdomen *Bowel sounds, masses, visceromegaly, and tenderness.*	**OK / Not OK** *Comment:*_____
Evaluation of lower extremities *Oedema and presence of arterial pulse*.	**OK / Not OK** *Comment:*_____
Inspection of the skin focus on lower extremities in people with diabetes.	**OK / Not OK** *Comment:* _____
Neurologic function *Reflexes*	**OK / Not OK** *Comment:*_____
Any orthopedic or medical condition that would limit exercise.	**YES / NO** *Comment:*_____
Ventricular tachycardia	**OK / Not OK** *Comment:*_____
ST elevation (+1.0 mm) in leads without diagnostic Q-waves (other than V_1 or aVR)	**OK / Not OK** *Comment:*_____
ST or QRS changes *such as excessive ST suppression >2mm horizontal or down sloping ST-segment depression*	**OK / Not OK** *Comment:*_____
Arrhythmias other than: *sustained ventricular tachycardia, including multiple PVCs, triplets of PVCs, supraventricular tachycardia, heart block, or bradyarrhythmias.*	**OK / Not OK** *Comment:*_____

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

Competed by: _____ **Date** _____

Physical examination (page 2 on reverse of page 1)

Exercise Stress Testing
Exercise Protocol: _____

Absolute indicators for terminating the Exercise Stress test:

Drop in blood pressure of >10mm Hg *from baseline blood pressure despite an increase in workload, when accompanied by other evidence of ischemia.*	**OK / Not OK** *Comment:* _____
Any form of chest pain or shortness of breath	**OK / Not OK** *Comment:* _____
Increasing nervous system symptoms (e.g. ataxia, dizziness or near syncope)	**OK / Not OK** *Comment:* _____
Technical difficulties monitoring ECG or blood pressure	**OK / Not OK** *Comment:* _____
Ventricular tachycardia	**OK / Not OK** *Comment:* _____
ST elevation (+1.0 mm) in leads without diagnostic Q-waves (other than V_1 or aVR)	**OK / Not OK** *Comment:* _____
ST or QRS changes *such as excessive ST suppression >2mm horizontal or down sloping ST-segment depression*	**OK / Not OK** *Comment:* _____
Arrhythmias other than: *sustained ventricular tachycardia, including multiple PVCs, triplets of PVCs, supraventricular tachycardia, heart block, or bradyarrhythmias.*	**OK / Not OK** *Comment:* _____
Fatigue, shortness of breath, wheezing, leg cramps, or patient develops discomfort.	**OK / Not OK** *Comment:* _____
Development of bundle-branch block or intraventricular conduction delay *that cannot be distinguished from ventricular tachycardia*	**OK / Not OK** *Comment:* _____
Hypertensive response Systolic blood pressure of > 250 mm Hg and / or diastolic pressure of >115 mm Hg	**OK / Not OK** *Comment:* _____

Comments:

_____
_____
_____

Adverse reaction to exercise: **YES / NO**

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

Competed by: _____ **Date** _____

Appendix 3 Absolute indicators for terminating an exercise test.

Absolute Indications

- Drop in blood pressure of >10mm Hg from baseline blood pressure despite an increase in workload, when accompanied by other evidence of ischemia.
- Any form of chest pain or shortness of breath
- Increasing nervous system symptoms (e.g. ataxia, dizziness or near syncope)
- Signs of poor perfusion (cyanosis or pallor)
- Technical difficulties monitoring ECG or blood pressure
- Subjects desire to stop
- Ventricular tachycardia
- ST elevation (+1.0 mm) in leads without diagnostic Q-waves (other than V_1 or aVR)
- ST or QRS changes such as excessive ST suppression (>2mm horizontal or down sloping ST-segment depression)
- Arrhythmias other than sustained ventricular tachycardia, including multiple PVCs, triplets of PVCs, supraventricular tachycardia, heart block, or bradyarrhythmias.
- Fatigue, shortness of breath, wheezing, leg cramps, or patient develops discomfort.
- Development of bundle-branch block or intraventricular conduction delay that cannot be distinguished from ventricular tachycardia
- Hypertensive response (systolic blood pressure of > 250 mm Hg and / or diastolic pressure of >115 mm Hg)

Adapted from the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Exercise Testing) (10).

Standard operating Procedure: MRC PETL 03

Exercise Training

MRC Muscle Performance and Exercise Training Laboratory
Standard Operating Procedure

SOP number:	MRC PETL 03
SOP full title:	Exercise Training
SOP category:	
SOP effective:	Review date:

SOP author signature: Date:

Dr Michael Trenell
Scientific Director, MRC Muscle Performance and Training Laboratory

SOP Reviewer signature: Date:

<name>, <title>

SOP approval signature: Date:

<name>, <title>

Background

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All participants in research projects being undertaken at the MRC Muscle Performance and Exercise Training laboratory must be protected from harm, undue risk and experience a friendly environment.

Purpose

To identify the process of exercise training following screening at the MRC Muscle Performance and Exercise Training Laboratory (defined in MRC PETL 01 and 02).

Scope

This SOP applies to all research participants undertaking exercise following screening at the MRC Muscle Performance and Exercise Training Laboratory. The SOP will define the procedures for an adverse event whilst exercising at:

1. Newcastle University Gymnasium
2. Participants local gymnasium
3. MRC Muscle Performance and Exercise Training Laboratory

Management of an adverse event

All exercise training studies performed with participants with chronic disease will be undertaken in an environment where support infrastructure is in place to manage an adverse reaction to exercise (such as collapse).

However, it should be reiterated that the major risks associated with exercise relate to having undiagnosed heart disease. Any individual reaching the stage of exercise training will have undergone screening for undiagnosed heart disease so the risk of an adverse event are low and are not likely to be serious. This is represented by the level of management of an adverse event.

Acute injuries will be managed by the senior physiotherapist team from the MRC Muscle Performance and Exercise Training Laboratory. The three locations for exercise training are:

Newcastle University Gymnasium
Any adverse event will be covered by the local procedures for management of an adverse event at the gymnasium.

Participants Local Gymnasium
Any adverse event will be covered by the local procedures for management of an adverse event at the gymnasium.

MRC Muscle Performance and Exercise Training Laboratory
Any adverse event will be covered by the local procedures of for management of an adverse event at CARU (the laboratory is based in CARU).

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Appendix 8

INFORMATION SHEET FOR RECRUITING VOLUNTEERS INTO A STUDY INVOLVING MRI SCANS

Newcastle Magnetic Resonance Centre
Newcastle University
Campus for Ageing and Vitality
Newcastle upon Tyne NE4 5PL

Email: scanner.bookings@ncl.ac.uk
Tel: (0191) 248 1150
Fax: (0191) 248 1151

Please could you pass this information to any team members who are involved in volunteer/patient recruitment into any studies that involve an MRI scan.

Volunteer safety is paramount; to this end we will safety screen all volunteers when they arrive at NMRC. In order to prevent an unnecessary booking it is advisable to screen your volunteers prior to recruitment into a study. There is a copy of the MR safety screening form attached, this can be used as a guide if you wish, and if the answer to any of these questions is **yes,** then please contact one of the NMRC radiographers before making an appointment.

The appointment time given is the time the volunteer will be on the scanner. We need up to 30 minutes with each volunteer prior to the scan in order to explain the procedure and to prepare them for the scan, and so please take account of this when arranging transport etc. If you also need to spend time with the volunteer prior to the scan then please take this into account also.

We ask that volunteers arrive prepared for an MRI scan, and where possible wearing no jewellery or any clothing with metal. All other metal, e.g. hair grips, must also be removed. We have a changing room with lockers should volunteers prefer to bring alternative clothing to wear for their scan. Your volunteer may also bring along a CD to listen to during the scan.

Please give each volunteer an NMRC appointment letter. This incorporates the patient/volunteer information sheet regarding their MR scan, and also a map and directions to NMRC.

If you have any queries or concerns, please do not hesitate to contact the NMRC radiographers.

Newcastle University

VOLUNTEER SAFETY QUESTIONNAIRE

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

Weight: _____ Height: _____

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

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

Do you have any of the following?

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

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

Are you wearing?

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

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

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

ALL metal worn or carried on your person must be removed

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

Volunteer's Signature: _____ Date: _____

Staff Signature: _____Date: _____

Appendix 10

Exercise Diary

Name: _____

Please fill in this diary after every exercise session:

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

Exercise Diary

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