EXERCISE AND INSULIN SENSITIVITY: INTERACTION WITH INTRAHEPATIC TRIGLYCERIDE AND HEPATOKINES

by

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Abstract

Insulin resistance is central to the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM). Intrahepatic triglyceride (IHTG), the primary feature of NAFLD, strongly predicts insulin resistance in the liver and peripheral (skeletal muscle and adipose) tissues. Hepatokines (e.g. fibroblast growth factor 21 (FGF21), leukocyte cell-derived chemotaxin 2 (LECT2), follistatin, selenoprotein P, and fetuin-A) are liver-derived proteins with capacity to exert endocrine effects and may potentially modulate the link between IHTG and peripheral insulin sensitivity/glycaemic control. Exercise is integral to the management of NAFLD and T2DM, with evidence suggesting that high-intensity exercise may provide the greatest benefits.

Chapter 4 of this thesis demonstrates that, in individuals without chronic metabolic disease, plasma concentrations of FGF21 and LECT2 are higher, and follistatin lower, in individuals with overweight or obesity compared with normal weight individuals. Furthermore, FGF21 and follistatin are transiently elevated for up to 6 h after acute aerobic exercise (60 min at 60% $\dot{V}O_2$ peak). The response of follistatin to acute moderate-intensity exercise is also present in individuals with impaired glucose regulation (Chapter 5), but the response of FGF21 is abolished. A single bout of low-volume high-intensity interval training has no effect on FGF21, follistatin or fetuin-A in individuals with dysglycaemia (Chapter 5). Chapter 6 demonstrates that six weeks of sprint interval training (SIT) is feasible for men with NAFLD and reduces IHTG despite no change in body weight. Peripheral insulin sensitivity tends to increase after SIT but hepatic insulin sensitivity and circulating hepatokines remain unchanged. Through meta-analyses, Chapter 7 confirms that exercise training reduces IHTG, even in the absence of weight loss. However, the magnitude of this effect is greater when weight loss occurs and benefits increase proportionally. Exercise training improves basal hepatic insulin sensitivity, but evidence in this area is currently limited (Chapter 7).

Collectively, the studies in this thesis demonstrate that some hepatokines may be sensitive to acute and chronic changes in energy metabolism. However, further evidence is required before definitive statements can be made. Exercise training, including SIT, has the potential to reduce IHTG in men with NAFLD, even in the absence of weight loss. However, the greatest benefits on IHTG will likely be elicited when exercise training is performed in combination with dietary energy restriction to elicit sustained reduction in body weight.

Key words: exercise, NAFLD, liver, insulin sensitivity, hepatokines, obesity

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Preface

Peer-reviewed publications

Chapter 4:

Sargeant JA, Aithal GP, Takamura T, Misu H, Takayama H, Douglas JA, Turner MC, Stensel DJ, Nimmo MA, Webb DR, Yates T & King JA. The influence of adiposity and acute exercise on circulating hepatokines in normal-weight and overweight/obese men. *Applied Physiology Nutrition and Metabolism*. (2018) **43**: 482-490.

Douglas JA, King JA, Clayton DJ, Jackson AP, Sargeant JA, Thackray AE, Davies MJ, Stensel DJ. Acute effects of exercise on appetite, *ad libitum* energy intake and appetite-regulatory hormones in lean and overweight/obese men and women. *International Journal of Obesity*. (2017) **41**: 1737-1744.

Chapter 6:

Sargeant JA, Bawden S, Aithal GP, Simpson EJ, Macdonald IA, Turner MC, Cegielski J, Smith K, Dorling JL, Gowland PA, Nimmo MA & King JA. Effects of sprint interval training on ectopic lipids and tissue-specific insulin sensitivity in men with non-alcoholic fatty liver disease. *European Journal of Applied Physiology*. (2018) **118**(4): 817-828.

Chapter 7:

Sargeant JA, Gray LJ, Bodicoat DH, Willis SA, Stensel DJ, Nimmo MA, Aithal GP, King JA. The effect of exercise training on intrahepatic triglyceride and hepatic insulin sensitivity: a systematic review and meta-analysis. *Obesity Reviews*. (2018) **19**: 1446-1459.

Conference presentations and published abstracts

Data from Chapter 4 were presented in the form of a poster at the *European Obesity Summit (EOS): Joint Congress of the European Association for the Study of Obesity and International Federation for the Surgery of Obesity and Metabolic Disorders – European Chapter 2016.* The accepted abstract was published with the following citation:

> Sargeant JA, Douglas JA, Aithal GP, Nimmo MA, Stensel DJ & King JA. The influence of acute moderate-intensity exercise on circulating fibroblast growth factor (FGF) 21 and fetuin-A concentrations in lean and overweight men. *Obesity Facts*. (2016) **9**(suppl 1): p86.

Data from Chapter 6 were presented in the form of posters at the *European Association for the Study of Liver, International Liver Congress 2017* and the *European Association for the Study of Obesity, European Congress on Obesity 2017*. The accepted abstracts for these presentations were published with the following citations:

> Sargeant JA, Bawden S, Simpson EJ, Kaviani M, Gowland P, Dorling JL, Nimmo M, Macdonald I, Aithal GP & King JA. Sprint interval exercise training reduces intrahepatic, visceral and subcutaneous abdominal fat despite no change in body weight, but has variable effects on whole-body insulin sensitivity. *Journal of Hepatology*. (2017) **66**(suppl 1): S429.

> Sargeant JA, Bawden S, Simpson EJ, Gowland P, Dorling JL, Nimmo M, Macdonald I, Aithal GP & King JA. The effects of sprint interval exercise training on hepatic and peripheral insulin sensitivity (IS), as well as intrahepatic triglyceride (IHTG), in men with nonalcoholic fatty liver disease (NAFLD). *Obesity Facts.* (2017) **10**(suppl 1): p210.

I was also invited to give an oral presentation of the data presented in Chapter 6 at the British Association for the Study of the Liver Basic Science Retreat 2018.

Invited meetings

I was successful in my application to attend the *European Association for the Study of Obesity, New Investigators United (NIU) Summer School 2016: "Obesity – A Multi-Systemic Disease"*. This meeting was held over 3 days in Cascais, Portugal and involved several structured and unstructured sessions designed to foster discussion between a small group of early-career researchers and international experts on various aspects of research and clinical practice related to obesity and obesity-related conditions.

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List of Abbreviations

Adipo-IR – adipose tissue insulin resistance index

ALT – alanine aminotransferase

AMPK – adenosine monophosphate-activated protein kinase

ANOVA – analysis of variance

AST – aspartate aminotransferase

AUC – area under the curve

BF% - body fat percentage

BMI – body mass index

BP – blood pressure

ChREBP – carbohydrate-responsive element binding protein

cJNK – c-Jun N-terminal kinase

CME – continuous moderate-intensity aerobic exercise

CKD – chronic kidney disease

coA – co-enzyme A (used with acetyl-, fatty acyl- and malonyl-coA)

CPT1/2 – carnitine palmitoyltransferase 1/2

CT – computed tomography

CV – coefficient of variation

CVD – cardiovascular disease

DAG - diacylglycerol

DGAT 1/2 – diacylglycerol acyltransferase 1/2

DNL – *de novo* lipogenesis

ECG – electrocardiography / electrocardiogram

EGP – endogenous glucose production

ELISA – enzyme-linked immunosorbent assay

ER – endoplasmic reticulum

FAT/CD36 – fatty acid translocase/CD36

FFM – fat-free mass

FGF21 – fibroblast growth factor 21

FM – fat mass

FoxO1 – forkhead box protein O1

FPI – fasted plasma insulin

FPG – fasted plasma glucose

FXR – farnesoid X receptor

GGT – gamma glutamyl transpeptidase

GLUT 1/4 – glucose transporter 1/4

HCC – hepatocellular carcinoma

HDL – high-density lipoprotein

HFD – high-fat diet

HIIT – high intensity interval training

HIRI – hepatic insulin resistance index

HISI – hepatic insulin sensitivity index

HOMA-IR – homeostatic model assessment of insulin resistance

HR – heart rate

HR_{max} – maximum heart rate

IFG – impaired fasted glucose

IGT – impaired glucose tolerance

IHTG – intrahepatic triglyceride

IPAQ – international physical activity questionnaire

IQR – interquartile range

IRS1/2 – insulin receptor substrate 1/2

LDL – low-density lipoprotein

LECT2 – leukocyte cell-derived chemotaxin 2

LPA – lysophosphatidic acid

LPL – lipoprotein lipase

LV-HIIT – low-volume high-intensity interval training

Matsuda ISI – Matsuda insulin sensitivity index

METs – metabolic equivalents of task

MRI – magnetic resonance imaging

NAFLD – non-alcoholic fatty liver disease

NASH – non-alcoholic steatohepatitis

NEFA – non-esterified fatty acids

NF-κB – nuclear factor kappa B

OGTT – oral glucose tolerance test

PAR-Q – physical activity readiness questionnaire

PI3k – phosphatidylinositol 3-kinase

PKC – protein kinase C (includes θ [theta] and ε [epsilon] isoforms)

PPAR – peroxisome proliferator-activated receptor (includes α [alpha] and γ [gamma] isoforms)

PPO – peak power output

RCT – randomised controlled trial

RER – respiratory exchange ratio

RPE – rating of perceived exertion

ScAT – subcutaneous adipose tissue

SD – standard deviation

SEM – standard error of the mean

SFA – saturated fatty acid

SIT – sprint interval exercise training

SeP – selenoprotein P

SREBP1c – sterol regulatory element binding protein 1c

TG – triglyceride

TGF – transforming growth factor

TZDs – thiazolidinediones

T2DM – type 2 diabetes mellitus

UPR – unfolder protein response

US - ultrasound

VAT – visceral adipose tissue

$\dot{V}CO_2$ – carbon dioxide production (volume of carbon dioxide produced per unit time)

VLDL – very low-density lipoprotein

$\dot{V}O_2$ – oxygen uptake (volume of oxygen utilised per unit time)

$\dot{V}O_2$ peak – peak oxygen uptake

WC – waist circumference

$\%EGP_{supp}$ – percentage suppression of endogenous glucose production (typically by low-dose insulin infusion)

^{1}H-MRS – proton magnetic resonance spectroscopy

95% CI – 95% confidence interval

CHAPTER 1

INTRODUCTION

Obesity is a chronic disease characterised by the excess accumulation of body fat, which is associated with several physical and metabolic consequences, and increases risk of disease-specific and all-cause mortality (Bray, 2004; Whitlock *et al.*, 2009; The Global BMI Mortality Collaboration, 2016; Heymsfield and Wadden, 2017). The prevalence of obesity has risen globally over recent decades (NCD Risk Factor Collaboration, 2016) and, with the simultaneous improvement in the management of infectious diseases, has become established as a leading health concern in modern societies (Heymsfield and Wadden, 2017). If current trends continue, it is predicted that, by 2030, there may be as many as three billion individuals worldwide that are overweight or obese (Kelly *et al.*, 2008). It is estimated that approximately 27% of the United Kingdom (UK) population are obese, many of whom are severely obese, and a further 35% are overweight (NCD Risk Factor Collaboration, 2016). The high prevalence of obesity, and the number of associated co-morbidities, make obesity treatment a substantial economic burden; one that is predicted to increase (Wang *et al.*, 2011; Bray *et al.*, 2016).

The pathogenesis of obesity is highly complex and obesity risk is determined by several modifiable and non-modifiable factors (Heymsfield and Wadden, 2017; Schwartz *et al.*, 2017). With rare genetic conditions aside, however, obesity is predominantly the result of prolonged energy surplus. Consequently, physical inactivity and excessive energy intake (as well as deleterious dietary composition) are important modifiable risk factors that are independently associated with increased obesity risk (Yumuk *et al.*, 2015). It is notable that reductions in physical activity over recent decades, particularly occupational physical activity, mirror the increased prevalence of overweight and obesity (Church *et al.*, 2011)

There has been increased recognition over recent years that adipose tissue is not simply a site for lipid storage but is, in fact, a metabolically-active endocrine organ (McGown, Birerdinc and Younossi, 2014). Accordingly, the metabolic consequences of obesity remain an area of growing interest and research. Obesity is associated with the increased risk of several metabolic co-morbidities, including type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), chronic kidney disease (CKD) and non-alcoholic fatty liver disease (NAFLD) (Bray *et al.*, 2016; Heymsfield and Wadden, 2017). As a result, the clinical management of obesity requires not only a focus on achieving and maintaining a healthy body weight, but must also consider the management of these associated conditions.

In recent years, there has been a rapid growth in the attention dedicated to NAFLD from both researchers and clinicians alike. In the UK, the number of hospital admissions due to NAFLD

has risen exponentially since 1998 and this is likely due to a combination of both increased obesity prevalence and increased clinical awareness (Williams *et al.*, 2014). NAFLD is characterised by the ectopic accumulation of lipid in the liver (hepatic steatosis), which occurs independently of excessive alcohol consumption (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018). Hepatic steatosis may occur in isolation, but it is the development of associated hepatic inflammation and/or fibrosis (non-alcoholic steatohepatitis; NASH), which substantially increases the risk of advanced liver complications (cirrhosis, hepatocellular carcinoma (HCC), and liver failure), as well as liver-specific and all-cause mortality (Rinella, 2015; Than and Newsome, 2015). NAFLD is now a leading chronic liver disease worldwide, with an estimated global prevalence of approximately 25% (Younossi *et al.*, 2016). Furthermore, NASH-related HCC is the most rapidly growing cause of liver transplantation and is set to surpass hepatitis C as the principal indication (Wong, Cheung and Ahmed, 2014; Rinella and Sanyal, 2016).

In the context of hepatic complications, therefore, the development of NASH represents an important aspect in the natural history of NAFLD. However, the clinical impact of hepatic steatosis *per se* should not be dismissed. Hepatic steatosis (measured as intrahepatic triglyceride; IHTG) is strongly associated with insulin resistance in several tissues, including skeletal muscle, adipose tissue and the liver (Korenblat *et al.*, 2008; Bril, Barb, *et al.*, 2017), and this has several implications for glycaemic control. Although the nature of these implications is highly complex, peripheral (skeletal muscle and adipose tissue) insulin resistance predominantly disrupts glucose uptake, whilst hepatic insulin resistance contributes to elevated endogenous glucose production (EGP) (Taylor, 2008). Unsurprisingly, therefore, NAFLD is commonly considered the hepatic component of the metabolic syndrome and is predictive of incident T2DM (Kotronen and Yki-Järvinen, 2008; Armstrong *et al.*, 2014; Byrne and Targher, 2015; Mantovani *et al.*, 2018). Insulin resistance is also heavily implicated in the pathogeneses of CKD and CVD (Laakso and Kuusisto, 2014; Artunc *et al.*, 2016; Xu and Carrero, 2017) and may, therefore, represent a central feature underlying the relationships between NAFLD and the myriad of obesity-associated conditions mentioned previously (Armstrong *et al.*, 2014; Byrne and Targher, 2015). It is notable that the leading cause of death in individuals with NAFLD is CVD (Targher, Day and Bonora, 2010; Targher *et al.*, 2016).

Several direct and indirect mechanisms have the potential to mediate the relationship between hepatic steatosis and peripheral insulin resistance (Byrne and Targher, 2015; Meex and Watt, 2017). One exciting, novel mechanism has emerged from the recent identification of several exclusively or predominantly liver-synthesised proteins, which have capacity to be secreted

into the circulation and exert endocrine effects in peripheral tissues. Consistent with the nomenclature of myokines and adipokines, these liver-secreted proteins have been termed "hepatokines" (Stefan and Häring, 2013; Takamura, Misu and Kaneko, 2016). Several of these hepatokines appear to be regulated by nutritional status (both acute and chronic), with hepatic steatosis implicated as a mediator of their synthesis and/or secretion (Meex *et al.*, 2015). Furthermore, some hepatokines have been shown to modulate peripheral insulin sensitivity or glycaemic control, attracting further interest into their clinical importance in NAFLD and other obesity-associated co-morbidities (Stefan and Häring, 2013; Meex *et al.*, 2015; Takamura, Misu and Kaneko, 2016).

There are currently no approved pharmacological treatments specifically for the treatment of NAFLD (Marchesini *et al.*, 2016; Rinella and Sanyal, 2016). There may be many reasons for this, but one important issue is the methodological difficulties and ethical considerations of using repeated liver biopsy (Festi *et al.*, 2013). Liver biopsy is highly invasive but, at present, regulatory bodies necessitate its use for the measurement of histological outcomes when testing pharmacological interventions. Several non-invasive imaging methods exist for the assessment of outcomes in NAFLD (Alkhouri and Feldstein, 2016; Bawden, Scott and Aithal, 2017). However, until suitable methods are developed, validated and approved by regulatory agencies for the measurement of histological outcomes (particularly hepatic inflammation and fibrosis), collecting sufficient experimental evidence to gain approval of pharmacological treatments in NAFLD will remain challenging (Rinella and Sanyal, 2016).

Consequently, lifestyle interventions remain the cornerstone of treatment in NAFLD (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018), and these are implemented with the primary aim of reducing body weight through the restriction of energy intake and increasing physical activity. The consumption of a more favourable dietary composition may also elicit independent benefits (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018). These guidelines (discussed in more detail within Chapter 2 of this thesis) provide generic goals for individuals, including weight loss targets and recommendations for both daily energy restriction and total weekly physical activity. Notably, however, they also fully endorse the use of a personalised approach, where each patient's treatment is discussed and agreed between a multidisciplinary team of healthcare professionals and, most importantly, in consultation with the patient themselves (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018). Holistic patient care means that agreed treatments should target the favourable modification of as many lifestyle risk factors as possible (diet, physical activity, smoking status etc.). However, specific interventions should

be tailored to patient preferences and circumstances (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018). This applies to the broader treatment approach (some individuals may favour energy restriction, whilst others prefer to focus on increased physical activity) as well as the specific details of the intervention employed. For example, when attempting to engage in more regular structured exercise, some patients may favour continuous moderate-intensity aerobic exercise (CME) training, whilst others may prefer high-intensity interval training (HIIT) or progressive resistance exercise. This individualised approach does, however, necessitate the development of several interventions that vary in nature and are supported by strong experimental evidence, to provide patients with multiple options to explore.

With this context in mind, the research reported in this thesis had three primary aims:

1. To examine the effects of acute exercise on several circulating hepatokines and their associations with anthropometric and circulating clinical biomarkers in individuals of different weight status and glycaemic control.
2. To test the feasibility and efficacy of sprint interval training (SIT) as a novel intervention for patients with NAFLD, particularly exploring its effects on IHTG and tissue-specific (hepatic and peripheral) insulin sensitivity.
3. To collate the existing literature and summarise the effects of exercise training on IHTG and hepatic insulin sensitivity, exploring the mediating influence of weight loss.

To do this, this thesis contains three laboratory-based experimental studies (Chapters 4, 5 and 6), which collectively target aims one and two, and concludes with a systematic review and meta-analysis (Chapter 7), which targets aim three.

Specifically, Chapter 4 investigates the influence of a single bout of CME on several candidate hepatokines in normal weight and overweight/obese men. Relationships between fasted plasma hepatokine concentrations, anthropometric measures and circulating cardiometabolic risk factors are also explored. Chapter 5 then extends these findings by conducting exploratory preliminary analyses on an ongoing clinical trial comparing the effects of acute CME or low-volume HIIT (LV-HIIT) on hepatokine responses in a patient group with dysregulated glucose metabolism. Chapter 6 presents the results of a 6-week SIT intervention in patients with NAFLD on hepatic steatosis (assessed by magnetic resonance spectroscopy) and hepatic and peripheral insulin sensitivity (determined by dual-step hyperinsulinaemic, euglycaemic clamp). Hepatokines were also measured in Chapter 6 to explore changes in fasted circulating concentrations with training. Finally, the systematic review and meta-analysis presented in

Chapter 7 systematically collates the available literature investigating the impact of exercise training on hepatic steatosis and hepatic insulin sensitivity in individuals with, or at high risk of, NAFLD. Importantly, inclusion criteria restrict eligible studies to those using the same techniques implemented in Chapter 6. Subgroup analyses and meta-regression in Chapter 7 explore the impact of exercise mode (aerobic, HIIT or resistance exercise) and intensity, intervention duration and the influence of associated weight loss on changes in hepatic steatosis.

The studies described in this thesis were core studies conducted within the National Institute of Health Research (NIHR) Leicester-Loughborough Diet Lifestyle and Physical Activity Biomedical Research Unit ("Leicester-Loughborough BRU"). The studies outlined in Chapters 4 and 5 were each designed and conducted in collaboration with other PhD students sponsored by the BRU. The data presented in Chapter 4 has been published (Sargeant, Aithal, *et al.*, 2018) and was selected by the editor as the featured article within its issue (Appendix I). Other outcomes of this trial have also been published (Douglas *et al.*, 2017). The study presented in Chapter 6, which was a collaborative project with the NIHR Nottingham Digestive Diseases BRU, has also been published (Sargeant, Bawden, *et al.*, 2018), as has the systematic review and meta-analysis presented in Chapter 7 (Sargeant, Gray, *et al.*, 2018).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Purpose and overview of this chapter

This chapter presents a detailed but succinct review of the literature that provides the underlying rationale for the studies presented in this thesis. The literature cited throughout is intended to be thorough, but is not an exhaustive list. Topics that are related but not specific to the focus of this thesis are mentioned in brief and appropriate published reviews are recommended for the interested reader.

This chapter starts by providing an overview of the pathogenesis of obesity and ectopic lipid storage before presenting an outline of the discovery, prevalence and natural history of NAFLD. This is followed by a more detailed account of the pathogenesis and treatment of hepatic steatosis, the central component of NAFLD, and its relationship with obesity and T2DM; focussing particularly on the role of physical inactivity and structured exercise training. This chapter concludes by presenting recent evidence concerning a selection of exclusively or predominantly liver-secreted proteins, termed 'hepatokines', and their potential implications for metabolic health as a novel mechanism mediating 'cross-talk' between the liver and peripheral tissues.

2.2 Obesity

2.2.1 Pathogenesis of obesity

The pathogenesis of obesity is highly complex, but in most individuals its development is ultimately governed by a chronic state of positive energy balance (Heymsfield and Wadden, 2017; Schwartz *et al.*, 2017). Energy balance is the sum of all processes related to energy intake and expenditure and, when intake exceeds expenditure, the excess energy is stored. Storage primarily occurs in the form of lipid (triglyceride; TG) accumulation in adipose tissue leading to increased body weight (Heymsfield and Wadden, 2017). Importantly, this increased storage is reversible and when a prolonged state of negative energy balance is achieved (through sufficient reductions in energy intake, increases in energy expenditure, or combinations of both) weight loss will occur (Heymsfield and Wadden, 2017). From an evolutionary perspective, the capacity of humans to store large amounts of energy in the form of lipid-rich adipose tissue is advantageous, conserving this energy during periods of surplus for times of relative famine (Schwartz *et al.*, 2017). However, in the modern environment, where energy is freely available and the requirements for physical exertion are reduced, this adaptation predisposes individuals to excessive weight gain.

The underlying factors that determine energy intake and expenditure in humans are numerous and interested readers are directed to published reviews that discuss the pathogenesis of obesity in depth (Van Der Klaauw and Farooqi, 2015; Heymsfield and Wadden, 2017; Schwartz *et al.*, 2017). A combination of genetic, developmental and environmental factors interact to determine obesity risk for a given individual. Many of these factors are fundamental to the pathogenesis of NAFLD and will be discussed in more depth in subsequent sections of this literature review.

2.2.2 Lipid storage in obesity

During chronic energy surplus, lipid accumulation occurs in several different sites (or 'compartments'), including both adipose and non-adipose tissues. The relative distribution of lipids across these tissues varies between individuals and, importantly, the associated metabolic risk for each compartment is not equal (Shen *et al.*, 2003; Blüher, 2013). Consequently, a given individual may have high levels of body fat yet remain relatively metabolically healthy in comparison to an apparently leaner person with a more adverse distribution of lipid storage (Thomas *et al.*, 2012; Blüher, 2013). In most individuals, the majority of lipid storage occurs in subcutaneous adipose tissue (ScAT) and this compartment is associated with the lowest metabolic risk (Tchkonia *et al.*, 2013). In a further level of complexity, the regional distribution of ScAT between upper body, lower body and abdominal sites may also be influential (Tchkonia *et al.*, 2013; Karpe and Pinnick, 2015). The visceral adipose tissue (VAT) compartment is a smaller absolute site for fat accumulation compared to ScAT, but is associated with a greater risk of metabolic dysregulation (Tchkonia *et al.*, 2013; Heymsfield and Wadden, 2017). Even in normal weight individuals, those with low ScAT and high VAT display greater metabolic risk compared to those with high ScAT and low VAT (Thomas *et al.*, 2012). In most individuals, when a state of chronic overnutrition and physical inactivity is sustained, the ability to preferentially store lipids within ScAT, becomes overwhelmed and an increased accumulation within VAT and other ectopic sites occurs. This may be further exacerbated by the development of adipose tissue dysfunction contributing to reduced storage capacity (Tan and Vidal-Puig, 2008; Blüher, 2013). Non-adipose sites of ectopic lipid storage include the vasculature, skeletal muscle, cardiac, renal and pancreatic tissues as well as, of particular relevance to this thesis, the liver (Szendroedi and Roden, 2009; Byrne and Targher, 2014). The ectopic accumulation of lipids in the liver is termed 'hepatic steatosis' and is the fundamental component of NAFLD.

2.3 Non-alcoholic fatty liver disease (NAFLD)

2.3.1 An introduction and historical overview

Hepatic steatosis is defined clinically as the presence of TG in more than 5% of hepatocytes or a liver fat content (intrahepatic triglyceride; IHTG) greater than 5.56%, depending on the measurement technique used (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018). Importantly, a diagnosis of NAFLD requires that hepatic steatosis occurs in the absence of excessive alcohol intake, viral infection and steatogenic medications, as well as other secondary sources. Consequently, most cases of NAFLD are fundamentally a result of deleterious lifestyle habits; usually a combination of excessive energy intake, adverse dietary composition and low physical activity (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018). Given that these are underlying components of positive energy balance, it is not surprising that the majority of NAFLD diagnoses occur in the context of overweight or obesity (Chalasani *et al.*, 2018). NAFLD describes a spectrum of conditions in which hepatic steatosis can either manifest in isolation (often referred to as 'benign' or 'simple' steatosis) or in combination with varying degrees of hepatic inflammation and/or fibrosis, known as NASH. In some cases, NAFLD may proceed to cirrhosis, HCC and liver failure, which may ultimately result in liver transplantation and/or death (Rinella, 2015; Than and Newsome, 2015; Marchesini *et al.*, 2016).

For decades, it was firmly believed that hepatic steatosis in the absence of viral infection was the result of excess alcohol consumption. Individuals who denied high alcohol intake were simply believed to be lying (Leslie, 2015). Then, in 1979, Adler and Schaffner reported findings from 29 overweight patients who, through individual interviews with three separate doctors, were considered to be no greater than "light social drinkers" (Adler and Schaffner, 1979). Liver biopsy samples from these patients all showed signs of hepatic lipid accumulation and patients were equally distributed between the categories of 'fatty liver', 'fatty hepatitis', 'fatty fibrosis' and 'fatty cirrhosis'. These findings led the authors to conclude that "obesity *per se* can lead to "severe liver damage" (Adler and Schaffner, 1979). A year later, Jurgen Ludwig and colleagues, from the Mayo Graduate School of Medicine, published similar findings from 20 individuals undergoing serial biopsy between the years of 1969 and 1979 (Ludwig *et al.*, 1980). These individuals were moderately obese but "denied alcohol abuse categorically", a claim that was supported by evidence from laboratory tests and consultation with relatives. Nonetheless, these patients presented with progressive histological changes that were compatible with alcoholic liver disease, including "striking fatty changes with evidence

of lobular hepatitis" and "evidence of fibrosis". Three individuals also developed cirrhosis (Ludwig *et al.*, 1980). As a result, Ludwig and colleagues were the first to propose the term NASH to describe this distinct medical entity (Ludwig *et al.*, 1980). For clinicians who remained doubtful, the rapid increase in the prevalence of fatty liver in children and adolescents provided further compelling evidence (Leslie, 2015).

2.3.2 Diagnosis

Exact definitions of hepatic steatosis differ subtly depending on the measurement technique used. Liver biopsy with subsequent chemical or histological assessment of tissue composition is the longest-standing method for the diagnosis of NAFLD. Using this method, hepatic steatosis is considered a fat percentage greater than 5% of liver volume or weight, or when more than 5% of hepatocytes visibly contain TG under microscopy (Fabbrini and Magkos, 2015). More recent advances in non-invasive imaging techniques, including ultrasound (US) and computed tomography (CT), has led to their routine use for early NAFLD diagnoses using similar diagnostic thresholds for liver fat content. Due to its high cost and invasive nature, liver biopsy is now reserved for when more progressive NAFLD is suspected, as it remains the only clinically approved method for the assessment of hepatic inflammation, fibrosis and cirrhosis. Circulating liver enzymes (alanine (ALT) and aspartate (AST) aminotransferases and gamma glutamyl transpeptidase (GGT)) are biomarkers of liver disease but are poorly correlated with IHTG and are insensitive to changes that occur (Charatcharoenwitthaya, Lindor and Angulo, 2012). However, most cases of NAFLD are diagnosed during clinical follow-up of abnormal liver function tests. Improvements in magnetic resonance techniques have allowed more sensitive non-invasive methods to be developed, namely proton magnetic resonance spectroscopy (^{1}H-MRS) and, more recently, magnetic resonance imaging (MRI). In a study of 2,349 individuals deemed to be at low risk of developing NAFLD, the 95th percentile of IHTG as assessed using ^{1}H-MRS was 5.56% (Szczepaniak *et al.*, 2005). Subsequently, this value has become an established threshold for the diagnosis of NAFLD when using this technique. Notably, however, this threshold criteria is not based on any relationship between IHTG and metabolic or clinical outcomes (Fabbrini and Magkos, 2015). For example, non-linear relationships have been reported between IHTG and tissue-specific insulin sensitivity, with suggestions that hepatic insulin sensitivity in particular (measured as percentage suppression of EGP) may be substantially impaired at IHTG content much lower than 5.56% (Bril, Barb, *et al.*, 2017). Furthermore, magnetic resonance procedures remain expensive and may not be practical in all individuals. As such, it is often reserved for clinical research studies.

2.3.3 General prevalence and common risk factors

NAFLD is now a leading cause of liver disease worldwide and the most prevalent in Western countries (Marchesini *et al.*, 2016; Cusi *et al.*, 2017). Its rapid rise in prevalence over recent decades make it an important health concern in both adults and children (Vernon, Baranova and Younossi, 2011; Than and Newsome, 2015). Prevalence estimates vary, primarily depending on the diagnostic technique used, but a recent meta-analysis of 45 studies with a total of over 8.5 million individuals reported the global prevalence of NAFLD to be approximately 25% [95% CI: 22.10 to 28.65%] (Younossi *et al.*, 2016). When analysed by region, data specific to Europe were similar (21 studies; 230,685 individuals; prevalence 23.71% [16.1 to 33.5%]), as were those in the single study from the UK (1,118 individuals; prevalence 26.39% [23.82 to 29.07%]) (Armstrong *et al.*, 2012). It is suggested that poor detection and referral of suspected NAFLD in at risk populations within primary care means that these research studies likely underestimate the true NAFLD prevalence (Armstrong *et al.*, 2012; Blais *et al.*, 2015).

The prevalence of NAFLD in normal weight individuals with no metabolic risk factors is between seven and 16% (Vernon, Baranova and Younossi, 2011; Younossi *et al.*, 2016). However, this is substantially increased in individuals who are obese and greater further in those with additional metabolic risk factors or co-morbidities (Rinella, 2015; Younossi *et al.*, 2016; Cusi *et al.*, 2017). NAFLD risk increases with body mass index (BMI) and waist circumference (WC) (Bedgoni *et al.,* 2005), and is prevalent in as many as 90% of severely obese individuals undergoing bariatric surgery (Machado, Marques-Vidal and Cortez-Pinto, 2006). Furthermore, approximately 50% of individuals with dyslipidaemia, defined as elevated circulating TG and reduced high-density lipoprotein (HDL), satisfy diagnostic criteria for NAFLD (Assy *et al.*, 2000; K.-T. Wu *et al.*, 2016). Conversely, dyslipidaemia is present in approximately 70% of patients with NAFLD (Younossi *et al.*, 2016). NAFLD is also tightly associated with insulin resistance and T2DM (Wanless and Lentz, 1990; Portillo Sanchez *et al.*, 2015; Younossi *et al.*, 2016; Cusi *et al.*, 2017) and these relationships are discussed in greater depth within subsequent paragraphs of this literature review (*2.3.6 Associations of NAFLD with insulin resistance and T2DM*).

The risk of NAFLD increases with age (Vernon, Baranova and Younossi, 2011; Attar and Van Thiel, 2013; Brea and Puzo, 2013), although this may be mediated by an increased likelihood of other metabolic risk factors (Frith *et al.*, 2009). NAFLD appears to be more common in men

but the reasons for this disparity are unclear and evidence is not unanimous (Vernon, Baranova and Younossi, 2011). Prevalence may also differ between ethnicities, with the risk of NAFLD greatest in Hispanic individuals and lower in white Caucasian and African Americans, respectively (Browning *et al.*, 2004; Vernon, Baranova and Younossi, 2011; Bril, Portillo-Sanchez, *et al.*, 2017). These differences may be partly accounted for by differences in the *PNPLA3* gene (Vernon, Baranova and Younossi, 2011) and, although a number of genes have been potentially implicated in the incidence and progression of NAFLD, variants in the *PNPLA3* and *TM6SF2* genes remains the most widely studied (Anstee, Targher and Day, 2013). Individuals who have the I148M and E167K variants in the *PNPLA3* and *TM6SF2* genes, respectively, have greater risk of both initial NAFLD incidence and more severe disease progression (Anstee, Targher and Day, 2013; Dongiovanni, Petta, Maglio, *et al.*, 2015).

It is important to consider that NAFLD and alcoholic liver disease may co-exist (Marchesini *et al.*, 2016) and that individuals with high alcohol intake may also have several unrelated metabolic risk factors that promote NAFLD (Adams, 2013; Marchesini *et al.*, 2016). Whilst distinguishing individuals with each of these diagnoses is common and understandable in a research environment, the adoption of this dichotomy may result in an underestimation of true NAFLD prevalence.

2.3.4 Natural history

The natural history of NAFLD is complex and not fully understood; not least because there appears to be distinct prognoses in individuals who maintain isolated steatosis and those that develop NASH (Rinella, 2015). Evidence from longitudinal studies is limited in number and varies in the use of diagnostic criteria and length of follow-up available. It is suspected, however, that approximately 20 to 25% of individuals with steatosis proceed to NASH and, of these, a further 20 to 40% will develop advanced fibrosis and/or cirrhosis over a 15-year period (Angulo, 2010; Caldwell and Argo, 2010). In the largest paired biopsy study to date, 108 patients were followed over a median of 6.6 years (McPherson *et al.*, 2015). Approximately 40% of patients showed worsening of NAFLD, 40% showed no change and 20% displayed improvements. Many of the patients that improved lost weight and presented with improvements in other metabolic factors (McPherson *et al.*, 2015). Furthermore, in a separate retrospective study of 420 NAFLD patients, approximately 12.5% died over a median follow-up of 7.5 years; a significantly lower survival rate than that of a matched control population (Adams *et al.*, 2005). Notably, this study did not distinguish individuals with simple steatosis

from those with NASH and it remains unclear if steatosis alone increases risk of mortality. It is well established, however, that all-cause and liver-related morbidity and mortality are substantially increased in individuals with NASH, compared to those with isolated steatosis and to the general population (Ekstedt *et al.*, 2006; Söderberg *et al.*, 2010; D. Kim *et al.*, 2013; Younossi *et al.*, 2016). Unsurprisingly, therefore, the progression to NASH represents a significant clinical outcome, particularly to hepatologists (Rinella, 2015; Than and Newsome, 2015; Marchesini *et al.*, 2016; Younossi *et al.*, 2016).

2.3.5 Hepatic steatosis vs. NASH

Given the evidence outlined above, it is not surprising that steatosis alone is sometimes considered to be a benign condition (Teli *et al.*, 1995; Caldwell and Argo, 2010). Whilst this may be partly true in the context of hepatic complications, this opinion is somewhat short-sighted with respect to wider multidisciplinary care (Bril and Cusi, 2017). The pathogenesis of NASH is highly complex and it seems an oversimplification to assume a dichotomous distinction between patients who are simply destined to develop NASH and those that are not (Peverill, Powell and Skoien, 2014). Instead, it is more likely that elevated IHTG is one of several factors that contribute to the increased risk of developing NASH, and that the threshold of resistance to this progression differs between individuals (Peverill, Powell and Skoien, 2014). Furthermore, whilst a number of non-invasive tools are in development, invasive liver biopsy remains the only way to definitively diagnose NASH and predicting individuals that proceed from isolated steatosis to NASH is difficult (Rinella, 2015; Marchesini *et al.*, 2016; Chalasani *et al.*, 2018). Therefore, until a better understanding of the pathogenesis of NASH is gained, along with more effective methods to identify high-risk individuals, the prevention and treatment of hepatic steatosis should be a clinical priority. Finally, the impact of NAFLD on metabolic health is not restricted to hepatic complications and the accumulation of IHTG is strongly associated with many extra-hepatic complications, including insulin resistance (Korenblat *et al.*, 2008; Gaggini *et al.*, 2013; Bril, Barb, *et al.*, 2017). Risk of metabolic co-morbidities, such as T2DM, CVD and CKD, is also increased in patients with NAFLD (Armstrong *et al.*, 2014; Musso *et al.*, 2014; Byrne and Targher, 2015; Sinn *et al.*, 2017). It is the associations between NAFLD, insulin resistance and T2DM that are of particular interest to this thesis and are, therefore, the focus of the subsequent paragraphs.

2.3.6 Associations of NAFLD with insulin resistance and T2DM

Elevated IHTG is strongly associated with insulin resistance and is an independent predictor of insulin action in the liver, adipose tissue and skeletal muscle (Fabbrini *et al.*, 2009). A study of 42 non-diabetic individuals (IHTG range: 0.7 to 45.5%) demonstrated strong linear correlations between IHTG and insulin sensitivity in the same three tissues (Korenblat *et al.*, 2008). A further study of 144 individuals with and without T2DM has confirmed these associations, but more complex non-linear relationships are suggested for hepatic and skeletal muscle tissues (Bril, Barb, *et al.*, 2017). Specifically, whilst a progressive reduction in adipose tissue insulin sensitivity is apparent with increasing IHTG, it is suggested that insulin sensitivity in the liver and skeletal muscle are maximally impaired at IHTG content of approximately 1.5 and 4%, respectively (Bril, Barb, *et al.*, 2017). Given these strong associations, it is not surprising that NAFLD is widely described as the hepatic manifestation of the metabolic syndrome (Buzzetti, Pinzani and Tsochatzis, 2016; Marchesini *et al.*, 2016).

Approximately two thirds of patients with T2DM have NAFLD (Williamson *et al.*, 2011; Portillo Sanchez *et al.*, 2015; Cusi *et al.*, 2017), whilst 22% of patients with NAFLD have diabetes (Younossi *et al.*, 2016). The study by Younossi and colleagues (Younossi *et al.*, 2016) does not distinguish between different classes of diabetes, but it is likely that the majority of these patients have T2DM. NAFLD also predicts T2DM incidence (Balkau *et al.*, 2010; Mantovani *et al.*, 2018) and is associated with a 2- to 5-times greater risk of disease development (Armstrong *et al.*, 2014). In a 14-year follow-up of 129 patients undergoing serial liver biopsies, 46% of those with steatosis alone developed T2DM, and this was even greater (~70%) in those with NASH (Ekstedt *et al.*, 2006). Importantly, the resolution of NAFLD is associated with a significant reduction of T2DM incidence (Sung, Wild and Byrne, 2013). Lastly, the presence of both NAFLD and T2DM is associated with greater severity of both conditions. Specifically, patients with both conditions are more likely to develop advanced NAFLD, are more insulin resistant and have more severe hyperinsulinaemia (Vernon, Baranova and Younossi, 2011; Loomba *et al.*, 2012; Lomonaco *et al.*, 2016).

2.3.7 Pathogenesis of hepatic steatosis

The liver is heavily involved in human lipid metabolism but, in healthy individuals, is not a preferred lipid storage site. A healthy 70 kg man with a body fat percentage of 20% may store as little as 125 g of fat in their liver (Leslie, 2015). This is clearly an oversimplification, however, and large individual differences are inevitable. That said, the liver has tremendous

potential to store large amounts of excess lipid when circulating concentrations are high. This adaptation may be important to avoid the potentially harmful effects of lipotoxicity in other tissues; a hypothesis that may be extended to explain the accumulation of intramyocellular lipids, which is common in individuals with obesity or associated conditions such as NAFLD (Watt, 2009). As mentioned previously, however, the sustained accumulation of lipid in the liver is not without consequence for most individuals. As little as 1.5% and 4% IHTG is associated with a substantial impairment in hepatic and peripheral insulin sensitivity, respectively (Bril, Barb, *et al.*, 2017). Furthermore, the 95th percentile of IHTG in a healthy adult population is only 5.56% (Szczepaniak *et al.*, 2005). Collectively, this evidence supports the proposition that hepatic steatosis may be an adaptive response with acute benefits, but when excessive or sustained over time, the accumulative effects become deleterious and pathogenic (Tilg and Moschen, 2010; Anstee, Targher and Day, 2013).

In its simplest form, the underlying cause of steatosis is an imbalance in the relationship between lipid supply and utilisation; when the supply of lipids to the liver overwhelms its ability to remove them, accumulation occurs (Fabbrini, Sullivan and Klein, 2010; Birkenfeld and Shulman, 2014; Brouwers *et al.*, 2016). As such, IHTG is determined by the balance between: a) fasted and post-prandial adipose tissue lipolysis, b) uptake of dietary lipids into the liver, c) hepatic *de novo* lipogenesis (DNL) of carbohydrate, d) hepatic lipid oxidation, and e) synthesis and export of lipid-rich very low-density lipoproteins (VLDL). These processes are presented in Figure 2.1. A more detailed account of these mechanisms at a molecular level, can be found in a thorough published review (Perry *et al.*, 2014).

In obese individuals undergoing liver biopsy for suspected NAFLD, the majority of IHTG can be accounted for by the delivery of circulating non-esterified fatty acid (NEFA) and hepatic DNL (Donnelly *et al.*, 2005). As such, in most cases, it is likely that changes in lipid supply are primarily responsible for the initial development of hepatic steatosis in NAFLD. Conversely, in accordance with the hypothesis that lipid storage as inert TG may be a protective adaptation, it may be that only when mechanisms of utilisation become overwhelmed and dysfunctional are associated co-morbidities, such as hepatic insulin resistance and NASH, developed (Tilg and Moschen, 2010). The factors underlying the development of hepatic steatosis and hepatic insulin resistance are outlined in subsequent paragraphs of this literature review. Readers interested in the pathogenesis of NASH are directed to a comprehensive review presenting the 'multiple parallel hits hypothesis' (Tilg and Moschen, 2010).

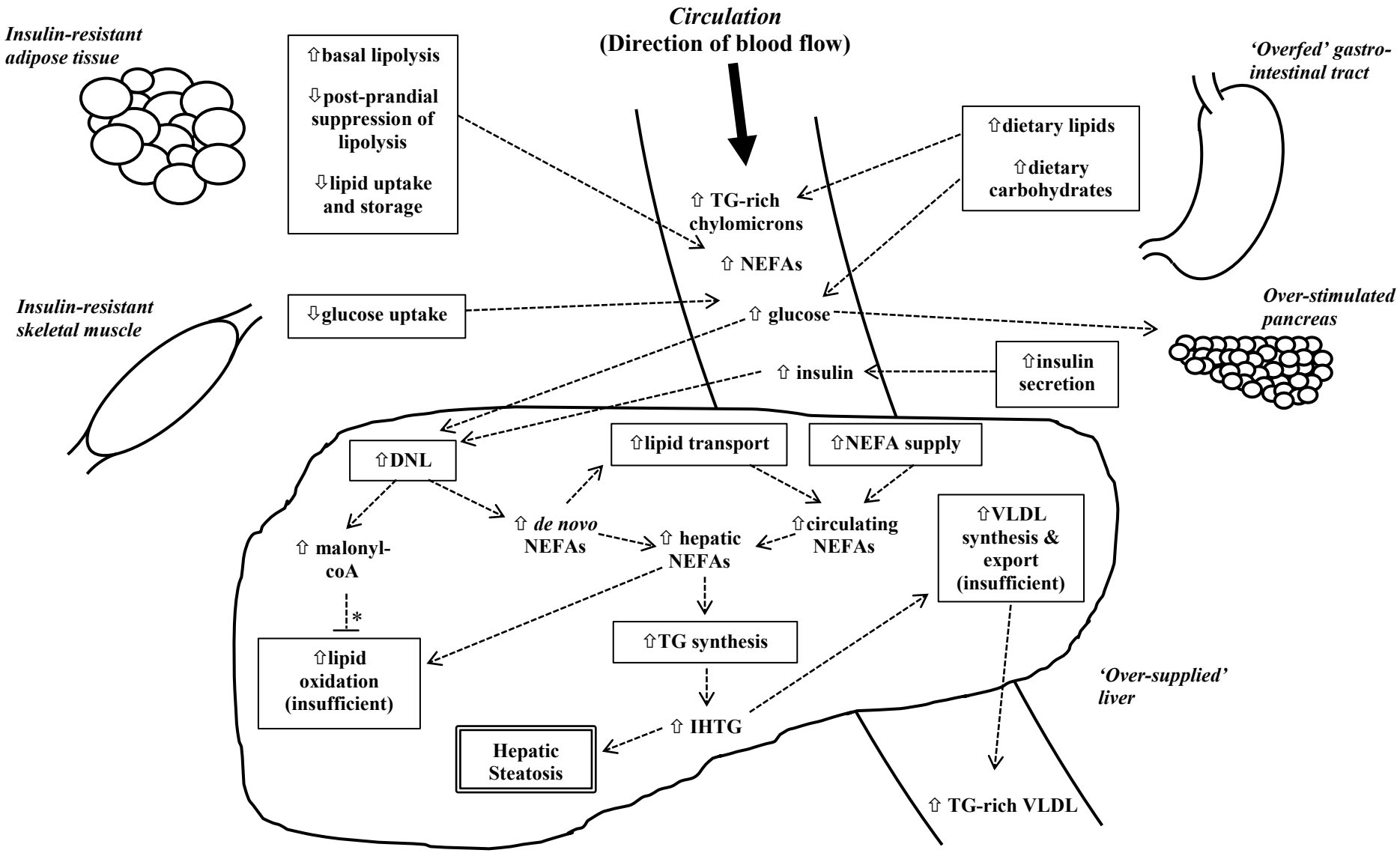


Figure 2.1 An overview of factors contributing to the pathogenesis of hepatic steatosis. Blood supply to/from the liver has been simplified for ease of interpretation. * Malonyl-coA inhibits lipid oxidation by reducing mitochondrial NEFA uptake. However, despite this, lipid oxidation is reportedly elevated in individuals with NAFLD compared to non-NAFLD controls. DNL: *de novo* lipogenesis; IHTG: intrahepatic triglyceride; NEFAs: non-esterified fatty acids; TG: triglyceride; VLDL: very low-density lipoprotein. Adapted from Fabbrini, Sullivan & Klein, 2010; Brouwers *et al*., 2016 and Perry *et al*., 2014.

2.3.7.1 Regulation of fatty acid uptake

NEFA uptake into the liver is determined by the combination of its delivery in the circulation and the capacity for transport into hepatocytes (Fabbrini, Sullivan and Klein, 2010). Hepatic NEFA uptake is not strictly regulated so increased circulating concentrations result in increased uptake, providing capacity for transport into the cell is not a limiting factor (Bradbury, 2006). Circulating NEFAs cross the cell membrane through specific transport proteins, including fatty acid translocase (FAT)/CD36 (Samuel, Petersen and Shulman, 2010). Circulating TG cannot be taken up directly, so it is first hydrolysed by hepatic lipoprotein lipase (LPL) to NEFA (Goldberg, Eckel and Abumrad, 2009). In individuals with obesity or NAFLD, hepatic expression of LPL is increased (Westerbacka *et al.*, 2007; Pardina *et al.*, 2009), whilst expression of FAT/CD36 correlates positively with IHTG (Greco *et al.*, 2008; Fabbrini *et al.*, 2009). Notably, FAT/CD36 expression is reduced following bariatric surgery and this reduction correlates with improvements in steatosis (Pardina *et al.*, 2017). In contrast, FAT/CD36 expression in adipose tissue is reduced in patients with NAFLD (Greco *et al.*, 2008; Fabbrini *et al.*, 2009) and the activation of adipocyte LPL in response to insulin is blunted in obese individuals compared to healthy controls (Sadur, Yost and Eckel, 1984). Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor coded by the *PPARG* gene, which regulates NEFA uptake into adipose tissue and the liver, as well as adipocyte differentiation (Schupp and Lazar, 2010). Individuals with dominant negative mutations of the *PPARG* gene in adipose tissue develop NAFLD (Savage *et al.*, 2003), whilst the deletion of hepatic *PPARG* in rodents protects against the development of hepatic steatosis (Matsusue *et al.*, 2003).

2.3.7.2 Adipose tissue lipolysis

In periods of relative fasting, when plasma insulin concentrations are low and glucagon high, TG stores in adipose tissue are broken down via lipolysis and NEFAs are released into the blood (Frühbeck *et al.*, 2014). Conversely, in the post-prandial state, when insulin levels are elevated, lipolysis is suppressed as part of the shift to a state of energy storage. Accordingly, when insulin resistance occurs in the adipose tissue, the regulation of lipolysis is impaired, resulting in elevated circulating NEFA under fasting conditions and a blunted post-prandial suppression (Frühbeck *et al.*, 2014). Consequently, NEFA supply to the liver is increased (Byrne, 2013).

Whole body rates of lipolysis are greater in obese compared with lean individuals (Fabbrini *et al.*, 2008). Furthermore, when matched for body fat, obese individuals with NAFLD have up to 2-fold greater lipolytic rates in adipose tissue than obese individuals with normal IHTG (Sunny *et al.*, 2011). The magnitude of suppression of lipolysis by insulin infusion is also negatively associated with IHTG (Korenblat *et al.*, 2008). In the fasted state, the majority of lipid supply to the liver is from the breakdown of ScAT (Fabbrini, Sullivan and Klein, 2010) and, in an intricate study using multiple lipid tracers, Donnelly and colleagues (Donnelly *et al.*, 2005) suggested that in individuals with NAFLD approximately 60% of IHTG originates from circulating, non-dietary NEFA. There is an additional supply of lipids originating from VAT but, contrary to the now widely-disputed 'portal vein hypothesis' (which suggested that hepatic steatosis was primarily caused by increased visceral lipids draining into the portal vein), the contribution of NEFA from VAT to total circulating NEFA remains low (albeit increased in obese (~20%) vs. lean (~5%) individuals) (Nielsen *et al.*, 2004).

Circulating NEFA concentrations during an oral glucose tolerance test (OGTT) are independently associated with IHTG (Holt *et al.*, 2006). This is primarily driven by a strong relationship between IHTG and post-prandial suppression of adipose tissue lipolysis. During both insulin infusion and an OGTT, the suppression of adipose tissue lipolysis is reduced in individuals with hepatic steatosis compared to either lean individuals or obese individuals with normal IHTG (Sanyal *et al.*, 2001; Seppälä-Lindroos *et al.*, 2002; Holt *et al.*, 2006). Only one of these studies found a relationship between NAFLD and circulating NEFA in the fasted state (Holt *et al.*, 2006), whilst several studies have shown no association (Sanyal *et al.*, 2001; Seppälä-Lindroos *et al.*, 2002; Tiikkainen *et al.*, 2002).

2.3.7.3 Dietary NEFA

Ingested lipids may contribute to IHTG as TG-rich chylomicrons pass into the fenestrations of the liver, are hydrolysed by LPL, and subsequently taken up into hepatocytes (Brouwers *et al.*, 2016). Approximately 15% of IHTG in individuals with NAFLD is suggested to originate from dietary sources (Donnelly *et al.*, 2005). Excessive energy intake and a high proportion of fat-rich foods combine to substantially increase the dietary intake of lipids and, as a result, the supply of lipid-rich chylomicron remnants delivered to the liver in the postprandial state. In addition, dysfunctional adipose tissue results in a 'spillover' of NEFA into the circulation, further increasing supply to the liver (Jacome-Sosa and Parks, 2014).

Within an isocaloric diet, macronutrient composition may be important, with suggestions that diets high in fat (and low in carbohydrate) may result in greater IHTG than those with low-fat, high-carbohydrate (Westerbacka *et al.*, 2005; Van Herpen *et al.*, 2011). However, hypo- and hypercaloric diets result in an increase or decrease in IHTG, respectively, with dietary composition seeming to be of less importance (Sevastianova *et al.*, 2012; Lecoultre *et al.*, 2013; Green and Hodson, 2014; Jensen *et al.*, 2018). Notably, however, the mechanisms by which diets high in fat or sugar promote hepatic steatosis may differ.

2.3.7.4 Hepatic de novo lipogenesis

De novo lipogenesis (DNL) is the conversion of glucose to palmitate, through various intermediates including acetyl-coA and malonyl-coA, for subsequent esterification to TG (Fabbrini, Sullivan and Klein, 2010). Hepatic DNL is regulated by several enzymes including fatty acid synthase (FAS) and diacylglycerol acyltransferase (DGAT) 1 and 2, as well as a range of transcription factors, such as sterol regulatory element binding protein 1c (SREBP-1c), carbohydrate-responsive element binding protein (ChREBP), farnesoid X receptor (FXR) and various peroxisome proliferator-activated receptor (PPAR) isoforms (Musso, Gambino and Cassader, 2009). In healthy humans, the contribution of DNL to hepatocyte-synthesised TG is low, accounting for less than 5%; although this was measured in TG-rich VLDL, not IHTG directly (Diraison, Moulin and Beylot, 2003). Conversely, in patients with NAFLD, the percentage of lipids in IHTG originating from DNL is suggested to be approximately 26% (Donnelly *et al.*, 2005).

Hepatic DNL is increased in individuals with NAFLD (Lambert *et al.*, 2014). Furthermore, it is independently stimulated by insulin (Ameer *et al.*, 2014) and glucose (Yamashita *et al.*, 2001) by activating SREBP1c and ChREBP, respectively (which are commonly increased in patients with NAFLD or T2DM). Obese individuals with hyperinsulinaemia have greater rates of hepatic DNL after five days of isocaloric high-fat diet (HFD) than insulin-sensitive lean and obese controls (Schwarz *et al.*, 2003). Furthermore, a three week period of high-carbohydrate overfeeding in obese individuals results in an increase of IHTG which correlates directly with increases in hepatic DNL (Sevastianova *et al.*, 2012). Notably, *de novo* synthesised NEFAs activate PPARα to further stimulate hepatic NEFA uptake (Chakravarthy *et al.*, 2005) which, in combination with impaired mitochondrial uptake and utilisation (mediated by inhibition of carnitine palmitoyltransferase (CPT) 1 by malonyl-coA), will further promote accumulation of IHTG (McGarry and Foster, 1980).

It is important to mention here the impact of skeletal muscle insulin resistance, which is also highly correlated with IHTG (Korenblat *et al.*, 2008). Mice lacking the skeletal muscle glucose transporter (GLUT)-4 develop severe hepatic steatosis and insulin resistance (Kotani *et al.*, 2004). Hyperinsulinaemia and hyperglycaemia, as a consequence of skeletal muscle insulin resistance, will further stimulate hepatic DNL as ingested carbohydrate is diverted away from skeletal muscle glycogen synthesis and towards the liver, where it is converted to palmitate and stored as IHTG (Petersen *et al.*, 2007; Flannery *et al.*, 2012). Fructose may be particularly important in this because the nature of its carbon phosphorylation means that it cannot be converted to glycogen and thus proceeds to DNL (Leclerq, 2013).

2.3.7.5 Hepatic lipid oxidation

Lipids are the preferred hepatic fuel in the fasted state (McGarry and Foster, 1980). Hepatic lipid oxidation occurs primarily in the mitochondria but also, to a lesser extent, in peroxisomes and microsomes (Fabbrini, Sullivan and Klein, 2010). The carnitine-dependent enzyme shuttle (involving CPT1 and CPT2 transporters along with carnitine acyltransferase) regulates transport of NEFAs into the mitochondria, where they are progressively shortened to produce acetyl-coA (Fabbrini, Sullivan and Klein, 2010). Acetyl-coA formed in this manner can then either provide a precursor for ketogenesis (to be exported and used as fuel in other tissues), or enter the tricarboxylic acid (TCA) cycle for β-oxidation (McGarry and Foster, 1980).

Somewhat paradoxically, the contribution of lipid oxidation to hepatic fuel metabolism is increased in hepatic steatosis and hepatic insulin resistance (Sanyal *et al.*, 2001; Iozzo *et al.*, 2010) and, in rodents, may be as high as 100% (Alves *et al.*, 2011). This may be explained as an adaptive response to increase the hepatic rate of lipid utilisation in attempt to limit excessive accumulation of IHTG. However, evidence in humans is limited. Gene expression of key enzymes in oxidative processes are increased in individuals with NAFLD compared to those with normal IHTG content, but hepatic expression of CPT1 is reduced along with mRNA of key components in mitochondria biogenesis (Greco *et al.*, 2008; Koliaki *et al.*, 2015). Mitochondrial respiration is, however, increased in obese individuals with NAFLD and correlates with IHTG (Sunny *et al.*, 2011), despite similar total mitochondrial content (Koliaki *et al.*, 2015). Furthermore, plasma ketone concentration, which can be used as an indirect measure to infer hepatic lipid oxidation, is increased in humans with NAFLD in some, but not all, studies (Sanyal *et al.*, 2001; Chalasani *et al.*, 2003).

2.3.7.6 Synthesis and export of lipid-rich very low-density lipoprotein (VLDL)

NEFAs that are not oxidised are esterified into TG and either stored as IHTG or exported in the form of TG-rich VLDL (Fabbrini, Sullivan and Klein, 2010). VLDL-TG secretion has repeatedly been shown to be greater in individuals with NAFLD compared to those with normal IHTG in both the fasted (Fabbrini *et al.*, 2008, 2009) and post-prandial states (Cassader *et al.*, 2001; Annuzzi *et al.*, 2004). However, there appears to be a ceiling effect whereby VLDL-TG secretion increases linearly throughout normal ranges of IHTG but may not increase further beyond IHTG of approximately 6%; suggesting that it may be an early adaptive response in attempt to limit IHTG accumulation (Fabbrini *et al.*, 2008).

2.3.8 Pathogenesis of hepatic insulin resistance

As mentioned previously, insulin resistance is heavily implicated in the development of hepatic steatosis and, in most individuals, strong associations exist between IHTG and insulin sensitivity in a range of tissues, including the liver (Korenblat *et al.*, 2008; Bril, Barb, *et al.*, 2017). Insulin resistance in skeletal muscle, hepatic and adipose tissues promotes steatosis, whilst steatosis, in turn, perpetuates insulin resistance. As such, the two are intricately linked within a myriad of conditions, which, in most cases, occur in the underlying context of obesity and physical inactivity.

The molecular mechanisms underlying insulin resistance are vast and complex and may differ subtly between tissues and amongst different insulin-dependent processes (Fabbrini, Sullivan and Klein, 2010). There is also increasing evidence that the steatotic liver in NAFLD may display selective insulin resistance whereby the regulation of glucose production is impaired, whilst that of TG synthesis is maintained (Cook *et al.*, 2015; Jelenik *et al.*, 2017). However, this literature will not be discussed in depth. Rather, the following paragraphs will provide an overview of the established concepts surrounding 'normal' and impaired insulin signalling in hepatic and skeletal muscle tissues, focussing on the regulation of circulating glucose. For simplicity, these processes will be presented in a linear manner, highlighting key signalling proteins. The reality is that many more steps of signalling are involved and their interaction is highly complex. For more information, interested readers are directed to excellent published reviews (Samuel, Petersen and Shulman, 2010; Samuel and Shulman, 2012; Perry *et al.*, 2014).

Circulating insulin binds to the insulin receptor at the cell membrane of hepatocytes and myocytes, causing tyrosine phosphorylation of insulin receptor substrates 1 and 2 (IRS1, IRS2)

(Samuel, Petersen and Shulman, 2010; Perry *et al.*, 2014; Shulman, 2014). In turn, IRS1/2 bind to and activate phosphatidylinositol 3-kinase (PI3K). In skeletal muscle, PI3K stimulates the translocation of GLUT4 to the cell membrane for the uptake of glucose from the circulation and subsequent conversion to intramuscular glycogen (Samuel, Petersen and Shulman, 2010; Perry *et al.*, 2014; Shulman, 2014). Insulin-mediated glucose uptake in the liver is much less than that of skeletal muscle, with evidence suggesting that signals from oral ingestion are required beyond the elevation of circulating glucose and insulin *per se* (Moore *et al.*, 2012). However, activation of hepatic PI3K by IRS1/2 results in the stimulation of Akt2 (also known as protein kinase B), which promotes glycogen synthesis and inhibits DNL, through the inhibition of glycogen synthase kinase 3 (GSK3) and forkhead box protein O1 (FoxO1), respectively (Samuel, Petersen and Shulman, 2010; Perry *et al.*, 2014; Shulman, 2014).

One established mechanism underlying insulin resistance is the activation of protein kinase C isoforms (PKCθ and PKCε in skeletal muscle and hepatic tissues, respectively), which translocate to the cell membrane and disrupt insulin signalling (Jornayvaz and Shulman, 2012; Birkenfeld and Shulman, 2014). Here, they cause phosphorylation of serine residues on IRS1 and IRS2 to impair downstream insulin signalling processes, including those outlined above (Samuel, Petersen and Shulman, 2010; Samuel and Shulman, 2012; Perry *et al.*, 2014). Insulin action may also be impaired through the activation of inflammatory pathways, mediated by nuclear factor-κB (NF-κB) (Fabbrini, Sullivan and Klein, 2010). Several factors associated with NAFLD mediate hepatic insulin resistance. The following paragraphs outline the impact of lipotoxicity, hepatic and peripheral inflammation and endoplasmic reticulum stress (Samuel and Shulman, 2012).

2.3.8.1 Lipotoxicity

In hepatocytes, NEFAs are progressively combined with glycerol-3-phosphate (a product of glycolysis) to form mono- (MAG), di- (DAG) and finally tri-acylglycerol (a term which is synonymous with triglyceride) (Samuel and Shulman, 2012). Whilst effective conversion and subsequent storage of NEFAs to TG may be considered a beneficial adaptation to energy surplus, it is an increase in many of these intermediates, particularly DAG, that is thought to have deleterious effects (Tilg and Moschen, 2010; Samuel and Shulman, 2012). In rodents, inhibition of hepatic DGAT2 (the enzyme which converts DAG to TG) results in a reduction of IHTG, a result that could be considered a beneficial shift in NAFLD risk/severity. However, this is accompanied by increased fatty acid oxidation, oxidative stress and liver injury

(Yamaguchi *et al.*, 2007). Notably, the knockdown of mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) 1, which catalyses the formation of lysophosphatidic acid (LPA) from fatty acyl-coA, results in a reduction of TG, and DAG, but does not prevent the development of hepatic insulin resistance, suggesting that DAG may not be the only lipid intermediate mediating this effect (Neschen *et al.*, 2005). Mice fed a 3-day HFD develop steatosis and hepatic insulin resistance, even before the development of obesity, peripheral insulin resistance or systemic inflammation (Samuel *et al.*, 2004, 2007). In these circumstances, both insulin-dependent activation of glycogen synthesis and suppression of EGP are impaired, and this can be attributed to an increased hepatic DAG, activation of PKCε and subsequent reduction in tyrosine phosphorylation of IRS1/2 (Samuel *et al.*, 2007). Importantly, knockdown of PKCε reduces HFD-induced hepatic insulin resistance despite similar hepatic DAG and TG content (Samuel *et al.*, 2007; Raddatz *et al.*, 2011). In humans with NAFLD, increased flux through TG-synthesis pathways results in increased production of lipid intermediates including LPA and DAG (Fabbrini, Sullivan and Klein, 2010; Samuel and Shulman, 2012). Hepatic PKCε and DAG are strong predictors of hepatic insulin resistance in human patients with NAFLD (Kumashiro *et al.*, 2011).

2.3.8.2 Local and peripheral inflammation

In rodents, selective activation of hepatic NF-κB induces hepatic inflammation and hepatic insulin resistance (Cai *et al.*, 2005). As adipose tissue expands, it can become infiltrated with macrophages, which consequently cause inflammation, insulin resistance and dysfunction (Samuel and Shulman, 2012). Recruited macrophages release cytokines and chemokines (including interleukin (IL) -6, tumor necrosis factor (TNF) α and monocyte chemoattractant protein (MCP) 1 into the circulation, which impair hepatic insulin signalling through the activation of hepatic NF-κB (Shoelson, Herrero and Naaz, 2007). Adipose tissue macrophage content and cytokine/chemokine production are increased in obese individuals compared to lean controls (Weisberg *et al.*, 2003) and in obese individuals with NAFLD compared to matched individuals with normal IHTG content (Kolak *et al.*, 2007). Lipid intermediates may also activate NF-κB to further contribute to hepatic insulin resistance beyond the direct effects of lipotoxicity mentioned above (Nagle *et al.*, 2007; Schenk, Saberi and Olefsky, 2008).

2.3.8.3 Endoplasmic reticulum stress

The endoplasmic reticulum (ER) is a cellular organelle that coordinates the post-translational synthesis, folding and trafficking of proteins (Fabbrini, Sullivan and Klein, 2010). Un- or mis-

folded proteins are identified by the ER for degradation by the proteasome (Samuel and Shulman, 2012). Under conditions of cellular stress, such as hypoxia or changes in substrate balance, unfolded proteins accumulate, resulting in impaired cellular function. In these circumstances, an unfolded protein response (UPR) is initiated in an attempt to restore normal function (Fabbrini, Sullivan and Klein, 2010). The UPR involves several cellular processes, including the activation of c-Jun N-terminal kinase (cJNK) which, in turn, activates NF-κB to induce insulin resistance (Özcan *et al.*, 2004). Conversely, experimental reduction of ER stress improves insulin action (Özcan *et al.*, 2006). Lipotoxicity is also a stimulus for the UPR in hepatocytes (Hotamisligil, 2010). Experimental research in humans is limited but individuals with NAFLD have been shown to have increased mRNA and/or activation of several proteins involved in the UPR (Puri *et al.*, 2008).

2.3.9 Treatment of NAFLD

2.3.9.1 Pharmacological therapies

No drug therapies are currently approved specifically for the treatment of NAFLD (Marchesini *et al.*, 2016; Rinella and Sanyal, 2016). As such, the use of pharmacological treatment in patients with NAFLD is restricted to off-label prescription (Marchesini *et al.*, 2016; Rinella and Sanyal, 2016), but due to the low prevalence of progressive liver complications in individuals with steatosis alone, this is usually reserved for individuals with progressive NASH or high risk of fibrosis (Singh *et al.*, 2015). Individuals with NAFLD may, of course, take prescription medications for the treatment of other associated co-morbidities such as T2DM, dyslipidaemia and hypertension. A handful of pharmacological agents have been tested for efficacy in the direct management of NASH and those that have reached phase II clinical trials will be outlined briefly in the following paragraphs, along with those more novel that show promise. Interested readers are directed to published reviews for additional information (Mazzella *et al.*, 2014; Ratziu, Goodman and Sanyal, 2015).

Thiazolidinediones (TZDs) are PPARγ agonists with insulin-sensitizing effects. The PIVENS trial investigated the effects of 2-year treatment with Pioglitazone in non-diabetic patients with NASH, comparing it with placebo or Vitamin E supplementation (Sanyal *et al.*, 2010). Pioglitazone successfully reduced all histological features of NASH except fibrosis and these benefits have been confirmed in a published meta-analysis (Mahady *et al.*, 2011). However, concerns exist regarding the side-effects of TZDs, which include weight gain, increased risk of bone fractures (particularly in women) and, rarely, congestive heart failure (Marchesini *et al.*,

2016). Other glucose-lowering agents show promise including incretin mimetics such as the GLP1-recpetor agonist, liraglutide, but large randomised controlled trials (RCTs) are required (Armstrong *et al.*, 2016). Metformin is an established first line treatment in T2DM, improving hepatic insulin sensitivity to elicit benefits on glycaemic control (Cusi, Consoli and DeFronzo, 1996). However, it appears to have no effect on histological features of NASH, including steatosis (Haukeland *et al.*, 2009).

One treatment arm of the PIVENS trial prescribed 800 IU/day of vitamin E and observed a reduction in steatosis, inflammation and lobular ballooning in 36% of patients (21% in the placebo group) (Sanyal *et al.*, 2010). Steatosis was reduced in 54% of patients compared to 31% of patients prescribed placebo. The TONIC trial demonstrated that vitamin E also reduces steatosis in children (Lavine *et al.*, 2011). However, its use has been limited following the results of a meta-analysis (19 clinical trials and almost 136,000 patients) suggesting that vitamin E at a dose of 800 IU/day is associated with increased risk of all-cause mortality (Miller *et al.*, 2005).

Obeticholic acid, a FXR agonist has also been investigated. Activation of FXR improves clamp-derived insulin sensitivity and decreases DNL (Mudaliar *et al.*, 2013). In the FLINT trial, obeticholic acid improved steatosis in individuals with NASH but LDL was also increased, raising concerns of an increased cardiovascular risk (Neuschwander-Tetri *et al.*, 2015). Issues of tolerability have also been reported (Marchesini *et al.*, 2016; Rinella and Sanyal, 2016). Notably, a rodent study has demonstrated reduced atherosclerotic risk following treatment with obeticholic acid, despite elevations in circulating LDL (Miyazaki-Anzai *et al.*, 2014).

Supplementation with polyunsaturated fatty acids (PUFAs) has been shown to reduce IHTG in early clinical trials (Argo *et al.*, 2015). However, despite its beneficial effects on insulin sensitivity and systemic inflammation giving clear rationale for expected benefits in NAFLD (Pettinelli *et al.*, 2009; Patterson *et al.*, 2012), phase II trials have failed to demonstrate histological improvements (Sanyal *et al.*, 2014; Dasarathy *et al.*, 2015).

There is also early evidence for the effective use of lipid lowering agents such as pentoxifylline (Van Wagner *et al.*, 2011; Zein *et al.*, 2011) and orlistat (Zelber-Sagi *et al.*, 2006; Harrison *et al.*, 2009). However, whilst cheap and well tolerated, the effects of pentoxifylline may be reserved for only a subset of individuals (Rinella and Sanyal, 2016). Statins are safe for use in patients with NAFLD but their direct effects on histological outcomes have not been investigated properly (Dongiovanni, Petta, Mannisto, *et al.*, 2015).

Part of the difficulty in gaining approval for pharmacological treatments in NAFLD is that regulatory bodies continue to demand the use of liver biopsy for the assessment of histological changes. This results in a number of practical difficulties, particularly regarding repeated testing of control groups in RCTs. It is hoped that with the ongoing development of non-invasive methods to assess hepatic inflammation, fibrosis and cirrhosis, these alternative methods may gain approval and in turn increase the number of phase II and III trials. Ultimately, however, the lack of liver-related complications associated with steatosis alone means that the recommended use of pharmacological treatment in individuals with non-advanced NAFLD (other than treatment of co-morbidities) is unlikely in the near future.

2.3.9.2 Bariatric and metabolic surgery

Bariatric, or metabolic, surgery remains an option for weight loss in individuals where other weight management approaches have failed (Marchesini *et al.*, 2016). A study in which 150 overweight/obese patients with T2DM were randomised to either continued medical therapy, gastric bypass or sleeve gastrectomy, has recently published 5-yr follow-up data (Schauer *et al.*, 2017). Both surgical interventions elicited reductions in body weight, HbA1c and lipid profile. Patients that underwent gastric bypass lost significantly more weight, but no differences between surgeries were reported for any other outcome (Schauer *et al.*, 2017). It has also been reported that surgical intervention reverses steatosis, NASH and possibly fibrosis one year after surgery in individuals with biopsy-proven NASH (Lassailly *et al.*, 2015). The median biopsy-derived steatosis (percentage of hepatocytes with evidence of steatosis) reduced from 60% (IQR 40 to 60%) at baseline to 10% (2.5 to 21.3%) at 1-year. Surgery is also reported to reduce liver enzymes and other outcomes of relevance to patients with NAFLD, including systemic inflammation and hypertension (Aguilar-Olivos *et al.*, 2016). A recent study in rodents has demonstrated that sleeve gastrectomy reduces IHTG and improves hepatic insulin sensitivity independent of weight loss (Gazala *et al.*, 2018). However, there are risks and expense associated with these procedures and they are not routinely recommended for individuals with NAFLD, particularly on the grounds of isolated steatosis alone. Studies investigating liver-specific mortality, liver transplantation and quality of life are required before this management approach is likely to change (Aguilar-Olivos *et al.*, 2016).

2.3.9.3 Lifestyle intervention

Given the evidence outlined above, lifestyle intervention, consisting of changes to diet and physical activity, remains the cornerstone of treatment in NAFLD and is mandatory for all

patients, regardless of disease severity (Marchesini *et al.*, 2016; Rinella and Sanyal, 2016). The underlying aims of this intervention are to reduce body weight and increase cardiorespiratory fitness (Rinella and Sanyal, 2016), as relatively small amounts of weight loss reduce IHTG and improve hepatic insulin sensitivity (Petersen *et al.*, 2005). Furthermore, physical fitness is inversely related with NAFLD risk (Zelber-Sagi, Ratziu and Oren, 2011). One notable exception is the unique group of individuals with 'lean NAFLD' in which weight loss is not a valid treatment goal. However, even in these patients, a healthy dietary composition and active lifestyle should be encouraged (Marchesini, Petta and Dalle Grave, 2016).

In a sub-analysis of patients enrolled in the Look AHEAD trial (a 12-month intensive lifestyle intervention aimed at reducing T2DM risk), participants completing the intervention lost significantly more weight, had greater reductions in IHTG and had lower risk of NAFLD progression compared to those who remained in standard care (Lazo *et al.*, 2010). Whilst at least 5% weight loss is associated with improvement in hepatic steatosis, greater reductions may be required to elicit other histological improvements (Promrat *et al.*, 2010; Vilar-Gomez *et al.*, 2015). In a 12-month uncontrolled community healthcare intervention, where 261 patients underwent paired liver biopsy, a dose-response relationship was apparent between the magnitude of weight loss and histological improvement. NASH resolution was observed in 26%, 64% and 90% of patients when weight loss of 5-7.5%, 7.5-10% and >10% was achieved (Vilar-Gomez *et al.*, 2015). Notably, only 30% of individuals randomised to the intervention (target energy restriction of 750 kcal per day and increased physical activity) achieved at least 5% weight loss. In a separate trial in individuals with NASH, weight loss >7% was associated with histological improvement regardless of allocation to lifestyle intervention or standard care groups (Promrat *et al.*, 2010).

Using hypocaloric diet alone, a 5% reduction in body weight over a few weeks is associated with a 25% improvement in IHTG, up to the resolution of 'healthy' levels (Patel *et al.*, 2015). In a large randomised trial, the composition (low-carbohydrate vs. low-fat) of a 6-month hypocaloric diet did not appear to have a substantial contribution to reductions in IHTG (Haufe *et al.*, 2011). However, over a shorter duration (two weeks) carbohydrate restriction may be more effective than general caloric restriction in reducing IHTG (Browning *et al.*, 2011), although evidence is limited. The Mediterranean diet, which is high in mono-unsaturated fatty acids (MUFAs) and fibre, and low in saturated fatty acids (SFAs), has been shown to reduce IHTG over six weeks in individuals with NAFLD, independent of weight loss (Ryan *et al.*, 2013). Furthermore, increases in IHTG as a result of overfeeding are attenuated in groups

consuming a diet low in SFAs compared to a high-SFA diet (Rosqvist *et al.*, 2014). The consumption of dietary fructose in the form of high-fructose corn syrup, the principal sweetening component of sugar-sweetened beverages, is associated with hepatic steatosis (Zelber-Sagi *et al.*, 2007; Maersk *et al.*, 2012), and the addition of fructose to overfeeding protocols exacerbates increases in IHTG (Sobrecases *et al.*, 2010). However, the consumption of fruit (which is also high in fructose) is considered safe and healthy for individuals with or at risk of NAFLD (Marchesini, Petta and Dalle Grave, 2016). Notably, however, a randomised trial in which participants consumed a diet high in either fructose or glucose under both isocaloric and hypercaloric circumstances (each for two weeks with a six-week washout between), reported significantly increased homeostatic model assessment of insulin resistance (HOMA-IR) by the high-fructose diet in the isocaloric period only (Johnston *et al.*, 2013). There were no other differences between the diets in either isocaloric or hypercaloric periods in relation to measures of adiposity (including IHTG) or clamp-derived indices insulin sensitivity.

Joint-guidelines from the European Associations for the Study of Liver, Diabetes and Obesity (EASL, EASD, and EASO) state the following components should be considered when designing a lifestyle intervention in the treatment of NAFLD (Marchesini *et al.*, 2016):

- Promotion of energy restriction with a target energy deficit of 500-1000 kcal/day.
- Minimum target weight loss of 7-10% initial body weight.
- Changes in dietary composition, if desired, according to the Mediterranean diet, whilst consumption of sugar-sweetened beverages should be avoided.
- Strict adherence to recommended weekly alcohol limits (< 14 and 21 units for women and men, respectively).

These are very similar to the joint-guidelines of the American College of Cardiology, American Heart Association and the Obesity Society for the management of overweight and obesity (Jensen *et al.*, 2014). Guidelines also promote the incorporation of physical activity or exercise, and the reduction of sedentary time, as follows:

- Completion of 150-200 $min \cdot wk^{-1}$ of moderate-intensity exercise, divided across three-to-five sessions.
- Gradual increase in physical activity with the aim of inducing an energy deficit of 400 $kcal \cdot day^{-1}$.

- Addition of resistance exercise to elicit musculoskeletal benefits (this may replace aerobic exercise in individuals for whom it may not be feasible, appropriate or well adhered to).
- Reduction in sedentary time and increase in daily steps (towards a target of 10,000 to 12,000 steps·day^{-1}).
- Avoidance of sustained periods of inactivity to lower lethargy and increase compliance.

All available guidelines for lifestyle interventions promote the use of individually tailored programmes and the setting of goals according to patient preferences (Jensen *et al.*, 2014; Marchesini *et al.*, 2016; Marchesini, Petta and Dalle Grave, 2016; Rinella and Sanyal, 2016). Cognitive behavioural therapies should be used to increase compliance and remove perceived barriers to weight loss and maintenance. Long-term evidence concerning the natural history of NAFLD following lifestyle intervention is lacking and no guide on the length of monitored weight maintenance exists (Marchesini *et al.*, 2016; Marchesini, Petta and Dalle Grave, 2016; Rinella and Sanyal, 2016). However, providing structured monitoring and support to individuals for as long as possible will likely improve adherence and this may translate to greater benefits.

2.3.9.4 Structured exercise training

The effects of exercise alone in the treatment of NAFLD have also been investigated. Chapter 7 of this thesis presents a systematic review and meta-analysis of the effects of exercise training on IHTG and hepatic insulin sensitivity in individuals with or at risk of NAFLD. As such, the following section of this literature review will remain brief.

The potential for exercise to induce large energy deficits is much smaller than that of caloric restriction (Marchesini, Petta and Dalle Grave, 2016). However, meaningful benefits of exercise on IHTG have been reported (Keating *et al.*, 2012; Katsagoni *et al.*, 2017). This is of clinical importance, particularly for individuals who may struggle to adhere to sustained periods of energy restriction (Montesi *et al.*, 2014). Exercise also elicits an array of wider cardiometabolic benefits, including increased fitness and insulin sensitivity, and reduced blood pressure (Kodama *et al.*, 2009; Garber *et al.*, 2011; James *et al.*, 2014; Colberg *et al.*, 2016). Both aerobic and resistance exercise have been shown to reduce IHTG and improve whole-body insulin sensitivity (Hallsworth *et al.*, 2011; Zelber-Sagi *et al.*, 2014; Cuthbertson *et al.*, 2016; Zhang *et al.*, 2016). Results from the STRRIDE-AT/RT trial suggests that the benefits of aerobic exercise may be greater than those of resistance training (Slentz *et al.*, 2011), but

any form of physical activity that replaces time spent sedentary is likely to be of benefit to most individuals with NAFLD (Marchesini, Petta and Dalle Grave, 2016). Importantly, exercise may elicit beneficial reductions in IHTG even in the absence of weight loss (Johnson *et al.*, 2009; Sullivan *et al.*, 2012), highlighting its important role in the management of NAFLD.

Given the mechanisms outlined previously that underlie the development of hepatic insulin resistance, it is feasible that exercise-induced reductions in IHTG and improvements in peripheral insulin sensitivity may correspond with improvements in hepatic insulin action. However, the effects of exercise training on hepatic insulin sensitivity in NAFLD are unclear. Stable or radioactive isotope-labelled glucose tracers are required to directly assess hepatic insulin sensitivity through the measurement of EGP in the basal and/or insulin stimulated states (Kim *et al.*, 2016). Basal rates of EGP appear to be unaffected by moderate-intensity aerobic or combined aerobic-plus-resistance exercise training in adults (Shojaee-Moradie *et al.*, 2007; Meex *et al.*, 2010; Hickman *et al.*, 2013; Cuthbertson *et al.*, 2016). However, when EGP is combined with insulin to form an index of hepatic insulin sensitivity one study reported a tendency ($P = 0.06$) for an improvement (Hickman *et al.*, 2013). Two studies by the same research group have also assessed the percentage suppression of EGP by low-dose insulin infusion before and after aerobic exercise training (Shojaee-Moradie *et al.*, 2007; Cuthbertson *et al.*, 2016), reporting contrasting findings. Hepatic insulin sensitivity was improved after six weeks of training despite no change in body weight or IHTG (Shojaee-Moradie *et al.*, 2007). However, 12 weeks of training, which elicited weight loss and significant reduction in IHTG, was not associated with improved percentage suppression of EGP (Cuthbertson *et al.*, 2016). None of these studies were formally powered to assess changes in hepatic insulin sensitivity and alternative forms of exercise, or different exercise intensities, have not been explored.

2.3.10 Mechanisms by which exercise may reduce IHTG

Prolonged negative energy balance reduces adipose tissue mass and body weight. After a sustained period of energy deficit, the greatest absolute changes in adipose tissue stores occur within subcutaneous sites, but substantial relative changes are also elicited in visceral and ectopic compartments including the liver (Lazo *et al.*, 2010; Promrat *et al.*, 2010; Vilar-Gomez *et al.*, 2015). Alongside energy restriction, regular exercise training can facilitate negative energy balance, weight loss and reductions in IHTG (Brouwers *et al.*, 2016). However, exercise training may also modulate the uptake and export of hepatic lipids beyond weight loss and changes in subcutaneous adipose tissue stores. These effects may underlie the benefits of

exercise that are reported independent of weight change (Johnson *et al.*, 2009; Sullivan *et al.*, 2012). Studies investigating these mechanisms in humans are limited, but the best available evidence is presented below.

2.3.10.1 Circulating NEFA in the fasted and post-prandial state

Six weeks of aerobic exercise training and six months of combined diet-plus-exercise training have each been shown to reduce basal rates of adipose tissue lipolysis in obese, post-menopausal women (You *et al.*, 2004; Shojaee-Moradie *et al.*, 2007). These benefits do not, however, always correspond to changes in IHTG (Shojaee-Moradie *et al.*, 2007). Furthermore, several studies report that fasted NEFA, which can be attributed to basal adipose tissue lipolysis, is unchanged by exercise training in patients with NAFLD, even when IHTG is reduced (Hallsworth *et al.*, 2011; Sullivan *et al.*, 2012; Cuthbertson *et al.*, 2016; Zhang *et al.*, 2016). As such, changes in post-prandial lipid metabolism may be more influential on the reduction of IHTG elicited by exercise training.

Shojaee-Moradie and colleagues reported that the suppression of adipose tissue lipolysis by insulin, measured using palmitate and glycerol tracers, was improved after six weeks of aerobic exercise training (Shojaee-Moradie *et al.*, 2007). Similar findings have been reported after 12 weeks of combined aerobic and resistance exercise in obese individuals with or without dysregulated glucose metabolism (Meex *et al.*, 2010). It is notable, however, that Meex and colleagues did not measure IHTG and Shojaee-Moradie *et al* reported that it was unaffected by exercise. Other studies have found that 12 weeks of aerobic exercise training in individuals with dysregulated glucose metabolism has no effect on the suppression of circulating NEFA after steady-state insulin infusion (Solomon *et al.*, 2009; Malin, Haus, *et al.*, 2013). In a study of obese, sedentary women, nine months of high- but not moderate-intensity aerobic exercise training improved the percentage suppression of NEFA by insulin, but these results could be attributed to a much lower suppression at baseline in the high-intensity group, compared to the moderate-intensity and control groups (DiPietro *et al.*, 2006). Similarly, 12 weeks of strength training in obese men has been reported to increase the percentage suppression of NEFA in response to insulin infusion. However, fasted circulating concentrations of NEFA were increased from pre- to post-training and the absolute concentrations in the insulin-stimulated state were unaffected (Polak *et al.*, 2005).

Collectively, the effects of exercise training on insulin-stimulated suppression of adipose tissue lipolysis in NAFLD are unclear. Whilst improved insulin-stimulated suppression of lipolysis

may be of theoretical benefit, these effects alone do not explain reductions in IHTG with exercise training. It should also be noted that reduced circulating concentrations of NEFA do not necessarily reflect a reduction in the rates of adipose tissue lipolysis and may instead be due to increased rates of clearance (Brouwers *et al.*, 2016). In cross-sectional studies using labelled lipid tracers, it has been suggested that increased aerobic fitness is associated with a greater uptake of NEFA into skeletal muscle. This finding complements the paradoxical phenomenon of high, yet healthy, intramuscular triglyceride in trained individuals (Iozzo *et al.*, 2004; Hannukainen *et al.*, 2007).

2.3.10.2 Dietary lipids and VLDL metabolism

The effects of exercise training on post-prandial hepatic lipid storage has not been explored directly so evidence is limited to inferences based on circulating fasted or post-prandial concentrations of VLDL or TG (Brouwers *et al.*, 2016). Six months of supervised aerobic exercise training in individuals with NAFLD elicited a reduction in fasted VLDL secretion, with no difference in the density or clearance of secreted particles (Alam *et al.*, 2004). Similar results have been demonstrated after an eight week programme of high-intensity interval training in obese, sedentary men (Tsekouras *et al.*, 2008). In contrast, 16 weeks of moderate-intensity aerobic training increased the clearance of large TG-rich VLDL particles, with no effect on rates of production (Shojaee-Moradie *et al.*, 2016), whilst another study reported that 16 weeks of aerobic exercise had no effect on VLDL metabolism (Sullivan *et al.*, 2012). Circulating TG concentrations in the fasted state are not reduced by exercise training in patients with NAFLD (Sullivan *et al.*, 2012; Hickman *et al.*, 2013; Cuthbertson *et al.*, 2016; Zhang *et al.*, 2016). Collectively, the effects of exercise on fasted VLDL metabolism in individuals with NAFLD are ambiguous at present. It is noteworthy, however, that an increased secretion of TG-rich VLDL would be required to explain the reductions in IHTG seen with exercise training and no study has reported this to date.

The circulating TG concentration in response to a meal is acutely attenuated by a single bout of moderate-intensity exercise for up to 24 hours and this may be mediated by increased clearance by skeletal muscle (Gill, 2004; Maraki and Sidossis, 2013). Cross-sectional studies have also reported that post-prandial triglyceridaemia is reduced in individuals that are physically active compared to sedentary individuals (Cohen, Noakes and Benade, 1989; Ziogas, Thomas and Harris, 1997). However, the benefits on post-prandial lipid metabolism with exercise may be restricted to the acute effects of the final exercise bout, rather than a true

training effect *per se* (Gill, 2004; Maraki and Sidossis, 2013). When assessments are made more than 24 hours after the final exercise bout of an exercise training programme, post-prandial TG concentrations are unchanged (Aldred, Hardman and Taylor, 1995; Herd *et al.*, 1998; Altena *et al.*, 2006). It could be speculated, however, that regular lowering of post-prandial TG after each session of an exercise training intervention may play a role in the reduction of IHTG, but further research is required to explore this hypothesis in detail.

2.3.10.3 De novo lipogenesis

No studies have directly explored the effects of exercise training on hepatic DNL in humans. However, in a series of studies in OLETF rats, key enzymes involved in several steps of DNL are reduced by exercise training, when employed either as a strategy to prevent/attenuate the accumulation of IHTG, or as a therapeutic intervention once steatosis has developed (Rector *et al.*, 2008, 2011, Linden *et al.*, 2014, 2015). These results are accompanied by reductions in circulating insulin and glucose, which are potent stimulators of DNL (Yamashita *et al.*, 2001; Ameer *et al.*, 2014). Although fasted concentrations of circulating glucose and insulin are unaffected by exercise training in individuals with NAFLD (Hallsworth *et al.*, 2011; Keating *et al.*, 2015; Cuthbertson *et al.*, 2016; Zhang *et al.*, 2016), peripheral insulin sensitivity is improved (Hickman *et al.*, 2013; Cuthbertson *et al.*, 2016). This will plausibly lead to reduced post-prandial concentrations of insulin and glucose, which may in turn reduce DNL. These effects could at least partially mediate reductions in IHTG by exercise training. Notably, acute exercise in lean, insulin-resistant individuals has been shown to improve the skeletal muscle response to insulin, reduce hepatic triglyceride synthesis and reduce hepatic DNL (Rabøl *et al.*, 2011). However, this hypothesis remains speculative and the effects of exercise training on markers of DNL in humans with NAFLD require detailed investigation.

2.3.10.4 Hepatic mitochondrial lipid oxidation

In the same series of studies in OLETF rats, the research group of Rector and colleagues reported an increase in complete oxidation of palmitate after exercise training, which was accompanied by an increase in key markers of hepatic mitochondrial lipid oxidation (Rector *et al.*, 2008, 2011, Linden *et al.*, 2014, 2015). These findings suggest that improved capacity for oxidative phosphorylation of NEFAs in the liver may play a part in post-exercise reductions in IHTG, but data in humans are lacking.

2.3.11 Relevance of exercise intensity in the management of NAFLD

Evidence suggests that exercise intensity may be an important parameter in determining the benefits of exercise training for individuals with NAFLD. In retrospective cross-sectional analyses, 813 individuals with biopsy-defined NAFLD were categorised according to whether they reported meeting United States guidelines for moderate- (150 min·wk^{-1}) or vigorous- (75 min·wk^{-1}) intensity physical activity (Kistler *et al.*, 2011). In these analyses, it was only individuals that met vigorous-intensity guidelines that had a significantly lower odds ratio of having advanced NAFLD (NASH and advanced fibrosis) (Kistler *et al.*, 2011). Notably, these individuals all had diagnosed NAFLD and the effects of exercise intensity on IHTG *per se* were not explored. In a recent study in rodents, 40 mice were randomised into one of four different 16-week interventions: 1) control (chow diet), 2) high-fat overfeeding, 3) high-fat overfeeding with moderate-intensity exercise training and, 4) high-fat overfeeding with high-intensity interval exercise training (Cho *et al.*, 2015). Exercise was performed during the final 8 weeks only. High-fat overfeeding resulted in the development of steatosis in all three groups but notably, the magnitude of IHTG accumulation was attenuated by exercise training in a step-wise manner according to exercise intensity (Cho *et al.*, 2015).

It may be important that completing exercise at a high-intensity does not come at the expense of total intervention volume. Individuals completing more than 250 min·wk^{-1} of moderate-to-vigorous physical activity during a 12 week lifestyle intervention demonstrated greater reductions in hepatic steatosis than those completing less than 250 min·wk^{-1} (Oh *et al.*, 2015). Whilst not unexpected, this underscores the importance of exercise volume, as well as intensity, within the management of NAFLD.

Collectively, these studies suggest that completing exercise of a higher intensity may elicit greater benefits on IHTG in individuals with NAFLD. Benefits may be greater still if a high training volume is maintained but something is better than nothing. A form of exercise training that may help support individuals to complete exercise of a greater intensity is HIIT.

2.4 High-intensity interval training (HIIT)

HIIT is a collective term describing several exercise protocols characterised by periods of high-intensity exercise interspersed with periods of rest or active recovery (Gibala, Gillen and Percival, 2014; MacInnis and Gibala, 2016). A variety of HIIT protocols exist which utilise a range of exercise intensities and durations. The most prominent of these protocols are ‘sprint

interval training' (SIT), comprising of 30-second bursts of 'all-out' activity (Burgomaster *et al.*, 2005), 'low-volume HIIT' (LV-HIIT), where individuals perform 10 x one-minute intervals at 'near-maximal' aerobic capacity (Little *et al.*, 2011), and 'aerobic interval training' (AIT), consisting of four-minute intervals at approximately 80-95% of maximum heart rate (HR_{max}) (Wisløff *et al.*, 2007). This is not an exhaustive list and variations of the above exist. Efforts also continue to try and identify the 'minimum dose' of interval-based exercise required to elicit benefits leading to additional protocols such as 'reduced exertion HIIT' ('REHIT') (Metcalfe *et al.*, 2012; Vollaard, Metcalfe and Williams, 2017). LV-HIIT and SIT are utilised in Chapters 5 and 6 of this thesis and, therefore, the following paragraphs will focus on the evidence surrounding these protocols and their relevance to the management of NAFLD.

2.4.1 Sprint interval training (SIT)

SIT is fundamentally modelled on performing repeated bouts of the Wingate Anaerobic test (Bar-Or, 1987). Participants complete 30-s intervals at maximal effort separated by periods of active recovery, usually 4.5 min. In 1998, it was demonstrated in young, recreationally active individuals that six weeks of SIT (three sessions per week) improved cardiorespiratory fitness, measured as peak oxygen uptake ($\dot{V}O_2$ peak), along with markers of skeletal muscle glycolytic and oxidative metabolism (MacDougall *et al.*, 1998). However, it was not until 2005 and the study by Burgomaster and colleagues (Burgomaster *et al.*, 2005), that research in this area started to gain momentum. In a series of studies, this research group demonstrated that SIT for as little as two weeks (total of six sessions) improved muscle oxidative capacity and endurance exercise performance (Burgomaster *et al.*, 2005; Gibala *et al.*, 2006). These effects remained after a 6-week intervention and benefits were comparable with those elicited by moderate-intensity aerobic exercise training (Gibala *et al.*, 2006; Burgomaster *et al.*, 2008). These findings have since been confirmed by a separate research group, who also reported that skeletal muscle microvascular function is improved by SIT (Cocks *et al.*, 2013). Notably, two weeks of SIT does not appear to improve cardiac output, suggesting that the early improvements in aerobic capacity may be limited to peripheral benefits in skeletal muscle (MacPherson *et al.*, 2011; Gibala, Gillen and Percival, 2014). Whilst the origin of the molecular signals responsible for these benefits are yet to be fully determined, peripheral improvements with SIT appear to be mediated by similar pathways to that of traditional moderate-intensity continuous training (Gibala, Gillen and Percival, 2014).

SIT may also elicit benefits of relevance to cardiometabolic health. This is important because the interval-based approach of HIIT may support individuals to acquire the benefits of exercise that may be otherwise unachievable through continuous steady-state activity (Kessler, Sisson and Short, 2012). As mentioned previously, the study by MacDougall and colleagues reported an increase in $\dot{V}O_2$ peak with six weeks of SIT (MacDougall *et al.*, 1998). This is important given the strong inverse relationship between cardiorespiratory fitness and mortality (cardiovascular and all-cause) (Blair *et al.*, 1989; Kodama *et al.*, 2009) These improvements in $\dot{V}O_2$ peak have since been confirmed with as little as two weeks of SIT in young healthy adults (Hazell *et al.*, 2010; MacPherson *et al.*, 2011; Astorino *et al.*, 2012), as well as in overweight or obese, but otherwise healthy, men (Whyte, Gill and Cathcart, 2010; Cocks *et al.*, 2015). A meta-analysis of RCTs has reported a moderate-to-large pooled effect size of SIT on $\dot{V}O_2$ peak (Gist *et al.*, 2014). The study by Hazell and colleagues (Hazell *et al.*, 2010) also reported a beneficial shift in body composition after six weeks of treadmill-based SIT with reductions in fat mass and increases in fat-free mass (FFM).

SIT has also been shown to improve glycaemic control. Whilst fasted circulating insulin and glucose are unaffected, two weeks of SIT improves whole-body insulin sensitivity in healthy young active men, determined using both oral-glucose tolerance test and hyperinsulinaemic, euglycaemic clamp (Babraj *et al.*, 2009; Richards *et al.*, 2010). Six weeks of SIT also improves OGTT-derived insulin sensitivity (the Matsuda insulin sensitivity index; ISI) in young, inactive individuals (Shepherd *et al.*, 2013). SIT is feasible for overweight or obese participants and, in accordance with evidence in normal-weight individuals, improves post-prandial insulin sensitivity and microvascular function to a similar extent as that of moderate-intensity training (Cocks *et al.*, 2015). Similarly, two weeks of SIT does not appear to elicit sustained benefits in HOMA-IR in overweight individuals (72 hours post-training), although acute benefits for up to 24 hours are apparent (Whyte, Gill and Cathcart, 2010).

2.4.2 Low-volume high-intensity interval training

Whilst SIT may be feasible in healthy overweight and obese volunteers (Whyte, Gill and Cathcart, 2010; Cocks *et al.*, 2015), it was suggested by Gibala and colleagues that SIT, as implemented in their initial studies, may not be safe or well-tolerated for all individuals (Little *et al.*, 2010). With these considerations in place, LV-HIIT was developed as a more feasible protocol for populations with, or at risk of, chronic disease (Little *et al.*, 2010). LV-HIIT retains a high exercise intensity, but one that is near to maximal aerobic capacity (approximately 80

to 95% of $\dot{V}O_2$ peak) as opposed to the supramaximal efforts of SIT. LV-HIIT is traditionally performed as 10 exercise intervals, each lasting one minute, separated by equal periods of low-intensity active recovery. Notably, these recovery periods were 75-seconds in the initial study by Little and colleagues (Little *et al.*, 2010), but reduced to 60 seconds thereafter (Hood *et al.*, 2011; Little *et al.*, 2011).

LV-HIIT has been shown to elicit similar benefits to those of SIT, in that $\dot{V}O_2$ peak, exercise performance and skeletal muscle mitochondrial function are all increased in young healthy individuals after as little as two weeks of training (Little *et al.*, 2010; Jacobs *et al.*, 2013). Furthermore, early benefits appear to be limited to skeletal muscle as cardiac output, haemoglobin concentration, haemoglobin mass and haematocrit are all reportedly unaffected (Jacobs *et al.*, 2013). In overweight but otherwise healthy individuals, two weeks and six weeks of LV-HIIT have each been shown to improve mitochondrial capacity which, over a longer intervention, is accompanied by reduced fat mass (FM) and increased FFM (Hood *et al.*, 2011; Gillen *et al.*, 2013). At present, however, the effects of LV-HIIT on glycaemic control are unclear. Hood and colleagues (Hood *et al.*, 2011) reported a reduced HOMA-IR in overweight individuals after two weeks of training, but OGTT-derived insulin sensitivity (Matsuda ISI) was unchanged after six weeks in a similar population (Gillen *et al.*, 2013). However, when patients with T2DM perform two weeks of LV-HIIT, glycaemic control, determined by continuous glucose monitoring, is reportedly improved (Little *et al.*, 2011). Over a 24-h period (measured 48 h after the final exercise bout), glucose concentrations in the interstitial fluid were reduced in response to standardised lunch and dinner meals. Furthermore, the mean glucose concentration over the whole 24-h period was lower after the LV-HIIT intervention. These benefits were accompanied by improved muscle mitochondrial capacity and higher protein content of GLUT-4 (Little *et al.*, 2011). LV-HIIT has also been shown to improve $\dot{V}O_2$ peak in patients with coronary artery disease (Currie *et al.*, 2013).

2.4.3 Perceptual benefits of SIT and LV-HIIT

One of the most prominent perceptual barriers to physical activity is a lack of time (Trost *et al.*, 2002; Kelly *et al.*, 2016). It has been suggested that SIT and LV-HIIT, along with other HIIT protocols, provide attractive and time-efficient strategies for individuals to incorporate structured exercise into their habitual routines (Gibala *et al.*, 2006; Hood *et al.*, 2011). A six-interval SIT session and 10-interval LV-HIIT session contain just three and 10 minutes of high-intensity exercise, respectively, which may be highly appealing to many individuals.

Furthermore, comparative studies by Gibala *et al* (2006) and Burgomaster *et al* (2008) reported that the benefits of SIT on exercise performance and muscle mitochondrial capacity were similar to traditional continuous moderate-intensity aerobic training, despite substantially lower weekly training time. It should be noted, however, that when accounting for recovery periods, warm-up and cool down (assuming five minutes each for the latter two) the absolute durations of SIT or LV-HIIT sessions are 40 and 30 minutes, respectively.

Another important barrier to physical activity is the perception that exercise is not enjoyable (Salmon *et al.*, 2003). It is plausible that some inactive individuals may find the prospect of completing intense but brief bursts of activity more appealing than that of prolonged CME. In turn, this may result in greater interest, motivation and, ultimately, adherence (Weston, Wisløff and Coombes, 2014). Sedentary young adults report greater enjoyment with LV-HIIT than when performing CME and more than 50% of participants in this study suggested that LV-HIIT was a form of exercise that they would consider if training unsupervised (Jung, Bourne and Little, 2014). In a separate study in which individuals were randomised to a six-week intervention of LV-HIIT or CME training, perceived enjoyment of LV-HIIT increased throughout the programme whereas enjoyment was either unaffected or declined with the CME programme (Heisz *et al.*, 2016). Interestingly, the rate of perceived exertion (RPE) during exercise training progressively decreases with intervals of shorter duration (Kilpatrick *et al.*, 2015), whilst protocols utilising 30- or 60-second intervals are more enjoyable than those using two-minute intervals (Martinez *et al.*, 2015). In a study comparing LV-HIIT and SIT directly, active individuals report no differences in measures of affect, including enjoyment, between the two protocols (Wood *et al.*, 2016). In overweight or obese individuals, measures of pleasure and enjoyment are no different when completing LV-HIIT compared to work-matched CME, despite a greater RPE (Little *et al.*, 2014). Patients with coronary heart disease or T2DM report that a general interval-based approach to exercise is more enjoyable than a session containing CME (Coquart *et al.*, 2008; Guiraud *et al.*, 2011). However, neither of these studies assessed the perceptions of LV-HIIT or SIT specifically.

Collectively, LV-HIIT and SIT appear to elicit physiological adaptations that are of relevance to cardiometabolic health, including cardiorespiratory fitness and insulin sensitivity. The magnitude of these effects are comparable to those elicited by CME and may occur with a lower total training volume. LV-HIIT and SIT may also be perceived by some individuals as more appealing, therefore representing an alternative strategy to engage in structured exercise that may be utilised within a personalised medicine approach. However, research in large

clinical trials with patient groups is required before definitive conclusions or wider recommendations can be made.

2.5 Hepatokines

The mechanisms underlying the relationship between hepatic steatosis and peripheral insulin resistance/glycaemic control are not fully understood (Takamura, Misu and Kaneko, 2016). Recent work has characterised the hepatic proteome as a group of 538 proteins, of which 168 have the capacity to be secreted into the circulation (Meex *et al.*, 2015). Many of these proteins have been shown to exert endocrine effects in peripheral tissues and as such, in analogy to 'myokines' and 'adipokines', have been termed 'hepatokines' (Stefan and Häring, 2011). With evidence that hepatokine secretion is modulated by hepatic steatosis (Meex *et al.*, 2015), it has been suggested that hepatokines may be one such mechanism that mediates the association between IHTG and peripheral insulin action/glucose homeostasis (Takamura, Misu and Kaneko, 2016).

2.5.1 Fibroblast growth factor (FGF) 21

The most widely studied hepatokine to date is fibroblast growth factor 21 (FGF21). While expressed in multiple tissues (including the liver, skeletal muscle and adipose tissue) (Fon Tacer *et al.*, 2010), circulating concentrations are predominantly liver-derived (Markan *et al.*, 2014). FGF21 has been implicated in a number of metabolic processes, including the regulation of glucose and lipid metabolism (Cuevas-Ramos, Aguilar-Salinas and Gómez-Pérez, 2012; Jung, Yoo and Choi, 2016).

The first evidence of FGF21 as a novel metabolic regulator was a series of experiments by Kharitonenkov and colleagues (Kharitonenkov *et al.*, 2005). In these (and subsequent) experiments, administration of FGF21 increased glucose uptake in rodent and human adipocytes, independent of insulin action (Kharitonenkov *et al.*, 2005; Xu, Lloyd, *et al.*, 2009), and this may was potentially mediated by increased expression of the GLUT1 transporter (Kharitonenkov and Adams, 2014). Transgenic rodent models with FGF21 knockout gain weight, have impaired glycaemic control and develop hepatic steatosis (Badman *et al.*, 2009; Kim *et al.*, 2015). Conversely, overexpression of FGF21 results in lower body weight, adiposity, IHTG and improved glucose regulation (Kharitonenkov *et al.*, 2005).

In rodents, short term (< 14 days) treatment with FGF21 elicits reductions in circulating glucose, TG and insulin, as well as improved glucose tolerance, whole-body glucose disposal and

hepatic insulin sensitivity (Kharitonenkov *et al.*, 2005; Berglund *et al.*, 2009; Xu, Stanislaus, *et al.*, 2009). These benefits are sustained following longer treatment (two to fifteen weeks) and are accompanied by reductions in body weight, body fat and IHTG, as well as enhanced insulin sensitivity in skeletal muscle and adipose tissue, and resistance to diet-induced obesity (Kharitonenkov *et al.*, 2005; Coskun *et al.*, 2008; Xu, Lloyd, *et al.*, 2009; Camporez *et al.*, 2013). Reductions in circulating TG and IHTG may be mediated by reduced adipocyte lipolysis and hepatic expression of SREBP1c, and increased hepatic lipid oxidation (Gimeno and Moller, 2014). Improved β-cell function has also been observed, but this finding has proved difficult to replicate in humans (Wente *et al.*, 2006; Stefan and Häring, 2013). In obese, diabetic humans, 28 days of treatment with an FGF21 analog (LY2405319) elicits an improved lipid profile (Gaich *et al.*, 2013). At higher doses (10 or 20 mg per day), body weight and circulating insulin are reduced compared to baseline, but these changes are not significantly different when compared to a placebo (Gaich *et al.*, 2013; Reitman, 2013).

Somewhat paradoxically, circulating FGF21 concentrations are, in fact, elevated in obese adults with both normal or dysregulated glucose metabolism (Zhang *et al.*, 2008; Chavez *et al.*, 2009; Mraz *et al.*, 2009). Furthermore, it is positively correlated with BMI and WC, each of which have been reported as independent predictors of circulating FGF21 in groups of healthy individuals and those with impaired glycaemic control or NAFLD (Zhang *et al.*, 2008; Li *et al.*, 2010; Mashili *et al.*, 2011; Cuevas-Ramos *et al.*, 2012). FGF21 is also elevated in patients with NAFLD (Dushay *et al.*, 2010; Li *et al.*, 2010), but while hepatic expression and circulating concentrations of FGF21 appear to increase with severity of steatosis (Li *et al.*, 2010; Yilmaz, Eren, *et al.*, 2010), it is unable to distinguish individuals with isolated steatosis from those with NASH (Dushay *et al.*, 2010).

Circulating FGF21 is also higher in individuals with T2DM compared to those with normal glucose tolerance, but this may be mediated by increased adiposity rather than glycaemic dysregulation *per se* (Chavez *et al.*, 2009; Mraz *et al.*, 2009; Chen *et al.*, 2011; Mashili *et al.*, 2011). FGF21 is, however, predictive of T2DM incidence (Chen *et al.*, 2011; Bobbert *et al.*, 2013) and correlates with a number of markers of glucose homeostasis, including fasted insulin and glucose, and 2-h glucose determined during an OGTT (Zhang *et al.*, 2008; Chavez *et al.*, 2009; Cuevas-Ramos *et al.*, 2010; Mashili *et al.*, 2011). FGF21 is also correlated with both whole-body and hepatic insulin sensitivity as measured using hyperinsulinaemic, euglycaemic clamp (Chavez *et al.*, 2009). It is suggested that increased circulating FGF21 in individuals with, or at elevated risk of, metabolic dysfunction may be a result of 'FGF21 resistance'

whereby, in analogy to insulin resistance, impairments in tissue signalling in response to FGF21 result in increased secretion (Fisher *et al.*, 2010; Potthoff, Kliewer and Mangelsdorf, 2012). Notably, obese mice have been shown to have impaired signalling responses to FGF21 infusion compared to lean controls (Fisher *et al.*, 2010). However, this hypothesis requires further investigation.

FGF21 is also positively correlated with self-reported physical activity (Cuevas-Ramos *et al.*, 2010, 2012) and $\dot{V}O_2$ peak is an independent predictor of circulating FGF21 concentrations (Taniguchi *et al.*, 2014). Furthermore, aerobic or combined aerobic and resistance exercise training for three to twelve weeks elicits a reduction in circulating FGF21 concentrations in young and elderly individuals at risk of metabolic disease (Yang, Hong, *et al.*, 2011; Scalzo *et al.*, 2014; Taniguchi *et al.*, 2016). In one of these studies, the reduction in FGF21 over five weeks of aerobic training was significantly positively associated with a reduction in IHTG, measured using ^{1}H-MRS (Taniguchi *et al.*, 2016).

A single run to exhaustion in healthy rodents results in elevated concentrations of FGF21, and this increase has been attributed to increased hepatic expression (K. H. Kim *et al.*, 2013). Circulating concentrations of FGF21 are also increased in humans after acute moderate- and high-intensity exercise in a stepwise manner, and these elevations are liver-derived (K. H. Kim *et al.*, 2013; Hansen *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016). Notably, this response may be blunted in obese individuals with impaired glycaemic control and completely abolished in individuals with overt T2DM (Slusher *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016).

The regulation of FGF21 expression is highly complex and not fully understood. It appears that its induction during states of fasting or overfeeding may be regulated by different mechanisms. Circulating NEFA, which occurs not only during fasting but also after strenuous exercise, may increase FGF21 expression in the liver and adipose tissue via activation of PPARα and PPARγ, respectively (Gälman *et al.*, 2008; Cuevas-Ramos *et al.*, 2012). Conversely, during energy surplus, increased circulating glucose may stimulate ChREBP to increase FGF21 expression (Iizuka, Takeda and Horikawa, 2009). FGF21 has also been shown to be regulated by the glucagon-to-insulin ratio (Hansen *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016) and this may explain, at least in part, the increase in circulating FGF21 with acute exercise. When individuals exercise under the conditions of a pancreatic clamp, in which a change in the glucagon-to-insulin ratio is prevented, circulating FGF21 is unchanged (Hansen *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016).

2.5.2 Leukocyte cell-derived chemotaxin (LECT) 2

LECT2 is a 16kDa secretory protein that is preferentially expressed in human liver cells and secreted into the circulation (Yamagoe, Mizuno and Suzuki, 1998). LECT2 was originally discovered when screening for novel neutrophil chemotactic proteins (Yamagoe *et al.*, 1996) and has been previously shown to exert anti-inflammatory and tumour-suppressive effects in the liver (Anson *et al.*, 2012). More recently, LECT2 has also been linked with obesity and its associated co-morbidities. Circulating concentrations of LECT2 are elevated with obesity and ultrasound-derived NAFLD (Okumura *et al.*, 2013), and correlate with BMI, waist circumference, HOMA-IR, FPI, TG, and the Matsuda ISI (Okumura *et al.*, 2013; Lan *et al.*, 2014).

A series of experimental studies by Lan and colleagues (Lan *et al.*, 2014) strongly implicates LECT2 in the development of obesity and insulin resistance. In liver biopsy tissue from 10 individuals with T2DM and seven lean controls, hepatic mRNA levels of LECT2 are significantly correlated with BMI, whilst gene expression of *LECT2* is increased in mice with HFD-induced obesity (Lan *et al.*, 2014). LECT2 appears to be highly sensitive to acute changes in diet and transitioning between periods of HFD and regular chow results in elevation and normalisation of LECT2 concentrations, respectively (Chikamoto *et al.*, 2016). Throughout these weight cycles, serum concentrations of LECT2 correlated with IHTG, but not adipose tissue weight (Chikamoto *et al.*, 2016).

LECT2 knockout (KO) attenuates increased body mass resulting from high-fat feeding (Lan *et al.*, 2014). Compared to wild-type controls, LECT2 KO mice also display greater insulin-stimulated Akt phosphorylation in skeletal muscle, but not in the liver or adipose tissue (Lan *et al.*, 2014). Accordingly, KO mice require greater glucose infusion rate to maintain euglycaemia during steady-state hyperinsulinaemia than wild-type controls, but the suppression of EGP is no different between groups (Lan *et al.*, 2014). In contrast, however, LECT2 treatment in cultured hepatocytes has been shown to induce steatosis, which is accompanied by significant reduction in hepatic IRS-1 phosphorylation and a tendency towards reduced Akt activation, suggesting a degree of hepatic insulin resistance (Hwang *et al.*, 2015). Interestingly, there are no differences in body mass, blood glucose, plasma insulin and circulating LECT2 between KO and wild-type mice after 60 days of starvation, suggesting that LECT2 may play a role in states of over- but not under-nutrition (Lan *et al.*, 2014). Very little is known about the influence of exercise on LECT2. However, LECT2 KO mice display greater

muscular endurance than wild-type controls (Lan *et al.*, 2014). Furthermore, when wild-type mice perform three hours of aerobic treadmill exercise, hepatic expression and protein content of LECT2, as well as circulating concentrations, are all reduced (Lan *et al.*, 2014).

Little is known about the molecular regulation of LECT2 in obesity or during exercise. However, LECT2 expression is negatively regulated by adenosine monophosphate-activated protein kinase (AMPK) in cultured hepatocytes and hepatic AMPK expression is decreased and increased during high-fat feeding and acute exercise, respectively (Lan *et al.*, 2014). Treatment of C2C12 myotubes with recombinant LECT2 leads to insulin resistance by increasing phosphorylation of cJNK, which in turn decreases insulin-stimulated Akt phosphorylation (Lan *et al.*, 2014).

2.5.3 Follistatin

Follistatin is a glycosylated protein and member of the transforming growth factor (TGF) -β superfamily. It was originally discovered in ovarian fluid but has since been shown to be expressed in several tissues including the liver and skeletal muscle (Phillips and de Kretser, 1998). Many of the functions of follistatin are facilitated through its natural inhibition of other TGFs, such as activin and myostatin (Phillips and de Kretser, 1998; Gilson *et al.*, 2009).

Serum follistatin concentrations are elevated in patients with NAFLD or T2DM (Yndestad *et al.*, 2009; Hansen *et al.*, 2013) and correlate with markers of insulin resistance including HOMA-IR, fasted plasma glucose (FPG) and 2-h glucose in some, but not all, studies (Wu *et al.*, 2012; Hansen *et al.*, 2013). Interestingly, one previous study reported lower plasma follistatin concentrations in individuals with T2DM compared with healthy controls (Ueland *et al.*, 2012). However, the activin to follistatin ratio was higher in these individuals. The potential that this ratio may be more important than concentrations of follistatin alone may explain discrepant findings.

Follistatin has been shown to promote muscle growth, via the negative regulation of both myostatin and activin (Gilson *et al.*, 2009; Yaden *et al.*, 2014). Greater skeletal muscle mass may increase glycaemic control via an increased absolute capacity for post-prandial glucose uptake. Rodent models in which follistatin was overexpressed display substantially increased muscle mass compared to wild-type controls (Lee and McPherron, 2001). Follistatin is also reported to increase proliferation of pancreatic β-cells. Overexpression of follistatin in the pancreas of diabetic mice results in increased β-cell mass, lower circulating glucose

concentrations, greater insulin production and, ultimately, greater lifespan (Zhao *et al.*, 2015). Administration of follistatin to rat β-cells *in vitro* lowers spontaneous cell death (Hansen, Rutti, *et al.*, 2016) but follistatin treatment of islet cells from healthy human donors had no effect on insulin secretion in response to low or high doses of glucose (Hansen, Rutti, *et al.*, 2016).

Given that a variety of tissues express follistatin, identifying which of these contribute substantially to circulating concentrations is difficult. Historically, the consensus has been that plasma follistatin is the sum of spillover from autocrine and paracrine actions within the many tissues in which it is expressed (Hansen and Plomgaard, 2016). However, a series of intricate studies has offered greater insight into the regulation of systemic follistatin at rest and during exercise (Hansen *et al.*, 2011; Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016). Circulating follistatin is increased following acute aerobic and resistance exercise, but expression and secretion from the exercising muscle are unaffected (Hansen *et al.*, 2011). This supports previous research reporting no changes in skeletal muscle expression of follistatin following acute or chronic exercise training (Jensky *et al.*, 2007, 2010; Besse-Patin *et al.*, 2014). Conversely, follistatin expression in the liver is increased with acute swimming exercise in rodents (Hansen *et al.*, 2011). In follow-up experiments (during which the splanchnic circulation was isolated by the insertion catheters into the brachial artery and hepatic vein), follistatin was constantly secreted from the livers of healthy human men at rest and this was increased 5-fold by acute aerobic exercise (Hansen, Rutti, *et al.*, 2016).

The altered secretion of follistatin by the liver reported by Hansen *et al* was regulated by changes in the glucagon to insulin ratio (Hansen, Rutti, *et al.*, 2016) and when acute exercise is performed under the conditions of a pancreatic clamp the increase in circulating follistatin is substantially attenuated (Hansen, Pedersen, *et al.*, 2016). Changes in the glucagon to insulin ratio can also be detected in the systemic circulation and a smaller shift in this ratio in individuals with T2DM may explain a blunted follistatin response to exercise (Hansen, Pedersen, *et al.*, 2016). The fact that the follistatin response is not completely abolished during pancreatic clamp indicates that other mechanisms are also responsible to some degree. Whilst these mechanisms remain unknown, evidence exists suggesting that hyperglycaemia, systemic low-grade inflammation and circulating NEFA are not responsible (Hansen *et al.*, 2013; Hansen, Pedersen, *et al.*, 2016). However, it has been speculatively suggested that activation of cyclic adenosine monophosphate (cAMP) by adrenaline may be an alternative mechanism that warrants investigation (Hansen, Pedersen, *et al.*, 2016).

2.5.4 Selenoprotein P (SeP)

SeP is a 60kDa glycoprotein encoded by the *SEPP1* gene (Lebensztejn *et al.*, 2016). It is primarily expressed in the liver and approximately 75% of circulating SeP is thought to be liver-derived (Burk and Hill, 2005, 2009). The role of SeP as a selenium supply protein is well established (Saito and Takahashi, 2002). More recently, however, potential roles within glucose homeostasis and insulin sensitivity have been revealed (Misu *et al.*, 2010). From the same analyses that identified the link between BMI and LECT2 (Lan *et al.*, 2014), an 8-fold greater hepatic expression of SeP was identified in individuals with T2DM along with a negative correlation between hepatic *SEPP1* mRNA and peripheral insulin sensitivity (Misu *et al.*, 2010). Circulating concentrations of SeP are also elevated in individuals with T2DM, compared with healthy controls, and positively correlate with FPG and HbA1c (Misu *et al.*, 2010). Positive associations have also been reported between circulating concentrations of SeP and BMI, WC, VAT, HOMA-IR, FPG and TG in individuals with normal and impaired glucose regulation (Yang, Hwang, *et al.*, 2011). When 120 non-diabetic Korean individuals (60 with ultrasound-defined NAFLD and 60 controls matched for age and sex) were divided into tertiles based on circulating SeP concentrations, NAFLD prevalence increased from the lowest to the highest tertile (H. Y. Choi *et al.*, 2013). Furthermore, a negative relationship has been reported between plasma concentrations of SeP and the liver attenuation index (LAI), a semi-quantitative method of measuring intrahepatic fat whereby lower LAI is indicative of greater steatosis (H. Y. Choi *et al.*, 2013).

Treatment of cultured hepatic cell lines and primary mouse hepatocytes with glucose or palmitate increases expression of *SEPP1* and SeP protein content (Misu *et al.*, 2010). Conversely, gene expression and protein content are reduced by insulin in a time- and dose-dependent manner (Misu *et al.*, 2010), which may be mediated by inhibition of FoxO1 (Speckmann *et al.*, 2008). Activation of AMPK by salsalate results in greater FoxO1 activation and reverses palmitate-induced upregulation of *SEPP1* (Jung, Choi, *et al.*, 2013).

When H4IIHEC hepatocytes and C2C12 myotubes are treated with SeP protein *in vitro*, insulin-stimulated phosphorylation of the insulin receptor and Akt are reduced, indicative of impaired insulin signalling. Accordingly, insulin-stimulated glucose uptake is decreased in myotubes, whilst glucose output from hepatocytes is increased (Misu *et al.*, 2010). Injection of purified SeP in C57BL/6J mice causes glucose intolerance and insulin resistance at a dose which increases circulating concentrations by a magnitude that is physiologically similar to the

difference between healthy and T2DM humans (Misu *et al.*, 2010). Phosphorylation of Akt was reduced in the liver and skeletal muscle of SeP-treated mice and this was associated with impaired hepatic suppression of EGP and peripheral glucose uptake, determined using hyperinsulinaemic euglycaemic clamp (Misu *et al.*, 2010). Glucose intolerance and insulin resistance are improved in SeP KO mice and they are protected against the deleterious effects of a high-fat, high-sucrose diet (Misu *et al.*, 2010). No study has investigated the effects of acute or chronic exercise on SeP.

The mechanisms by which SeP disrupts hepatic and peripheral insulin signalling remains an area of investigation. The phosphorylation of AMPK and acetyl-coA carboxylase (ACC) is increased in the livers of SeP KO mice (Misu *et al.*, 2010), whilst SeP treatment in cultured hepatocytes inhibits phosphorylation of these proteins. Furthermore, co-treatment with AICAR, a known activator of AMPK, prevents SeP-induced reductions in Akt phosphorylation (Misu *et al.*, 2010). It is of note, however, that the phosphorylation of AMPK in skeletal muscle is unaffected by SeP administration, suggesting that tissue-specific mechanisms may exist (Misu *et al.*, 2010).

2.5.5 Fetuin-A

Fetuin-A, also known as α_2 Heremans-Schmid glycoprotein, is a 64kDa phosphorylated glycoprotein that is primarily expressed in, and secreted from, the liver (Denecke *et al.*, 2003). Fetuin-A secretion is modulated by IHTG (Meex *et al.*, 2015). Single nucleotide polymorphisms in the human *AHSG* gene (which codes for fetuin-A) are associated with susceptibility to T2DM (Siddiq *et al.*, 2005; Andersen *et al.*, 2008), whilst fetuin-A KO mice display improved insulin sensitivity (Mathews *et al.*, 2002). Circulating fetuin-A is increased in obese and morbidly obese individuals when compared with healthy controls (Brix *et al.*, 2010; Ismail *et al.*, 2012) and, when participants with varying degrees of glycaemic control are grouped into quartiles based on circulating fetuin-A concentrations, BMI progressively increases from the lowest to highest quartile (Ix *et al.*, 2006; Xu *et al.*, 2011; Dutta *et al.*, 2014). Fetuin-A has also been shown to correlate with $\dot{V}O_2$ peak and is significantly greater in low compared to highly active individuals (Jenkins *et al.*, 2011).

Circulating fetuin-A is increased in patients with NAFLD when compared with healthy controls and NAFLD has been shown to be a strong predictor of fetuin-A, even after adjustment for age, sex, and other metabolic risk factors (Yilmaz, Yonal, *et al.*, 2010; Haukeland *et al.*, 2012; von Loeffelholz *et al.*, 2016). Fetuin-A has also been shown to correlate positively with

IHTG determined using ^{1}H-MRS or histological analysis of liver biopsy tissue (Stefan *et al.*, 2006; Kantartzis *et al.*, 2010; von Loeffelholz *et al.*, 2016). Interestingly, this increase with NAFLD is reported in individuals with normal and impaired glycaemic control (Ou, Yang, *et al.*, 2012).

Fetuin-A is also increased in individuals with T2DM versus non-diabetic controls (Ishibashi *et al.*, 2010; Song *et al.*, 2011; Dutta *et al.*, 2014). It has also been reported as an independent predictor of incident T2DM, even after adjustment for appropriate confounding variables (Ix *et al.*, 2012; Stefan *et al.*, 2014). Studies have also reported a stepwise increase in fetuin-A from normal glucose tolerance, through impaired fasted glucose (IFG) or impaired glucose tolerance (IGT) to overt T2DM (Ishibashi *et al.*, 2010; Ou, Yang, *et al.*, 2012; Dutta *et al.*, 2014; H.-T. Wu *et al.*, 2016). Fetuin-A is also significantly greater in insulin resistant obese individuals, compared with insulin-sensitive controls matched for age and adiposity (Klöting *et al.*, 2010). It should be noted that the insulin resistant group in this study also had significantly greater VAT but, unfortunately, IHTG was not measured. Independent associations have also been identified between fetuin-A and HOMA-IR, as well as peripheral insulin sensitivity measured using hyperinsulinaemic, euglycaemic clamp (Song *et al.*, 2011; Xu *et al.*, 2011; Kaess *et al.*, 2012; Stefan *et al.*, 2014).

Fetuin-A was first reported in 1989 as a strong natural inhibitor of insulin receptor tyrosine kinase in rat hepatocytes, a finding that was consequently confirmed in humans (Auberger *et al.*, 1989; Srinivas *et al.*, 1993). Fetuin-A promotes an inflammatory profile in mouse and human adipocytes and monocytes, and in the adipose tissue of mice (Hennige *et al.*, 2008; Dasgupta *et al.*, 2010). Fetuin-A attenuates lipogenic pathways in adipose tissue via inhibition of the PPARγ signalling pathway and promotion of lipolysis (Dasgupta *et al.*, 2010), and serves as an adaptor protein for the activation of Toll-like receptor 4 (TLR4) to induce inflammatory signalling and insulin resistance (Pal *et al.*, 2012). It has also been shown to inhibit GLUT-4 translocation in C2C12 myotubes via inhibition of the insulin receptor (Dasgupta *et al.*, 2010; Goustin, Derar and Abou-Samra, 2013; Malin, Mulya, *et al.*, 2013). Finally, incubation of HepG2 cells and rat hepatocytes with fetuin-A promotes steatosis, which is associated with increased expression of SREBP-1c (Jung, Youn, *et al.*, 2013).

Positive energy balance increases circulating concentrations of fetuin-A (Lin *et al.*, 1998; Samocha-Bonet *et al.*, 2014). Treatment of HepG2 cells and rat hepatocytes with palmitate increases the expression, synthesis and secretion of fetuin-A and this is dependent on NF-κβ

(Dasgupta *et al.*, 2010). Treatment of HepG2 cells with glucose also increases expression of *AHSG* (Takata *et al.*, 2009). In humans, fetuin-A tends to increase after 48 hours low-dose (30 $mL \cdot h^{-1}$) intralipid infusion (Hussey *et al.*, 2014). ER stress may mediate both glucose and palmitate-induced responses (Ou, Wu, *et al.*, 2012).

Circulating fetuin-A is reduced by weight loss resulting from combined diet and exercise (Stefan *et al.*, 2006; Reinehr and Roth, 2008), energy restriction alone (Blüher *et al.*, 2012; K. M. Choi *et al.*, 2013; Baldry *et al.*, 2017) and bariatric surgery (Brix *et al.*, 2010). Moderate-intensity aerobic exercise, with or without resistance training, failed to reduce fetuin-A in obese, healthy individuals or those with NAFLD or T2DM (Mori *et al.*, 2008; Schultes *et al.*, 2010; Yang, Hong, *et al.*, 2011; Cuthbertson *et al.*, 2016). This is despite a significant reduction in body weight, WC and BF%. In healthy sedentary men, 12 weeks of CME is associated with a mean 20% reduction in circulating fetuin-A (but this was not statistically significant) (Oh *et al.*, 2017). Continuous exercise of higher intensity (85% HR_{max}) does elicit reductions circulating fetuin-A over as little as seven days, when exercise is performed each day (Malin, Mulya, *et al.*, 2013). This beneficial reduction is sustained when similar exercise was performed five days per week over the course of 12 weeks (Malin *et al.*, 2014). However, the lack of control group and the short duration between the final exercise session and post-training assessments (16-18 h) are important limitations to these studies. Nonetheless, these interesting data provide rationale to further investigate the effects of exercise on fetuin-A. No studies have investigated the effects of acute exercise.

CHAPTER 3

GENERAL METHODS

The following chapter provides details of procedures that are, with the exception of magnetic resonance and dual-step hyperinsulinaemic, euglycaemic clamp procedures, common to two or more experimental studies contained within this thesis. Each experimental chapter also contains a separate methods section that outlines further details unique to that study.

3.1 Ethical approvals and participant recruitment

Studies involving human participants (Chapters 4, 5 and 6) were conducted with full ethical approval from an appropriate governing body. Chapters 4 and 5 obtained approval from the East Midlands NHS research ethics committee and were sponsored by the University Hospitals of Leicester NHS Trust. Chapter 6 was sponsored by Loughborough University and approved by its research ethics committee (human participants sub-committee). Chapter 6 also gained subsequent approval by the research ethics committee of the University of Nottingham. Confirmation of approval for each study can be found in Appendix II. All studies were performed in accordance with the Declaration of Helsinki (World Health Organisation, 2013). Accordingly, all participants gave informed, written consent having been provided with all study information in writing and verbally, and having had the chance to discuss these with members of the research team.

In all experimental chapters, participants were recruited by word-of-mouth and poster advertisement. Posters were distributed around local venues at which they would be visible to potential participants. Posters were also delivered to individual addresses within chosen postcodes via Royal Mail and distributed on social media platforms. Participants that had taken part in previous studies and given consent to be contacted in future were also contacted when they were identified as being potentially eligible. Furthermore, short online press releases were issued by Loughborough University and/or the University of Leicester. In Chapters 4 and 5 (which gained NHS ethical approval) participants were also recruited through local primary care services and supported by the NIHR Clinical Research Network. General practice surgeries that volunteered to participate were provided with information about the study including participant eligibility criteria. They were also provided with pre-screening packs, which were posted to individuals that they identified as being potentially eligible. These packs contained more detailed study information and a contact details form, along with a pre-addressed envelope so that interested individuals could return this information to the research team. Individuals who returned these packs were then contacted directly to discuss the study further and, if they remained interested, the formal recruitment process was initiated.

3.2 Participant pre-screening

All participants completed pre-participation screening to assess their suitability for exercise testing/training according to the standard operating procedures and risk assessment (SOP/RA) of the NIHR Leicester-Loughborough BRU. These procedures (detailed in Appendix III) were developed in accordance with common best practice and guidance set out by the European Association for Cardiovascular Prevention and Rehabilitation (Borjesson *et al.*, 2011). The specific components of screening that participants undertake differ according to the intensity of exercise for which participants are being screened, defined according to the metabolic equivalents of task (METs) associated with the exercise to be performed. All studies within this thesis include exercise greater than 3 METs and, therefore, participants were screened according to the 'moderate to vigorous' exercise pathway. This pathway is outlined below.

Briefly, participants were first categorised as 'active' or 'inactive' according to self-reported weekly physical activity, assessed using the short version of the International Physical Activity Questionnaire (IPAQ; www.ipaq.ki.se [accessed 03/12/2017]; Appendix IV). 'Active' participants were those that reported performing at least 20 min of vigorous exercise on three or more days per week. These individuals completed the physical activity readiness questionnaire (PAR-Q; www.csep.ca/en/publications [accessed 03/12/2017]; Appendix V) and when all questions were answered 'no', they were cleared for exercise testing. If one or more questions were answered 'yes', participants were stratified as 'low', 'moderate' or 'high' risk. All inactive individuals were also stratified in this manner. Stratification was based on the results of a face-to-face screening session conducted by a healthcare professional (research nurse, senior cardiac nurse or general practitioner) and within this session, participants provided a medical history, underwent a physical exam, gave a fingertip capillary blood sample, were assessed for 10-year CVD risk (via the 'SCORE' algorithm; see Appendix VI), and received a resting electrocardiogram (ECG). When no clear risk factors were identified, participants were considered 'low' risk and were cleared for exercise testing. Participants were considered 'high' risk when any of the following were identified:

- Diagnosis of chronic disease
- BMI > 35 $kg \cdot m^{-2}$
- 10-year risk of CVD ≥ 10%
- Strong family history of CVD in first degree relatives < 50 years of age
- Elevated total (> 8 $mmol \cdot L^{-1}$) or LDL-cholesterol (6 $mmol \cdot L^{-1}$)
- Elevated blood pressure (>180/110 mmHg)

Participants who did not meet criteria for either 'low' or 'high' risk categories were considered 'moderate' risk and, along with 'high' risk individuals, underwent an exercise ECG that was supervised by a qualified member of clinical staff. According to the NIHR Leicester-Loughborough BRU SOP/RA, only high-risk individuals were required to undergo exercise ECG screening within a clinical setting. However, within the studies contained in this thesis, all exercise ECGs were performed in a clinical setting with access to a resuscitation team and post-arrest management. Provided there were no contraindications to exercise, participants were cleared for further exercise testing/training.

3.3 Anthropometrics

Participant height was measured to the nearest 0.1 cm using a vertical stadiometer (Seca Ltd, Germany), whilst body weight (to 0.01 kg) and total body fat percentage (BF%) (to 0.1 %) were measured using a segmental body composition analyser (BC-418, TANITA Europe BV, Amsterdam, the Netherlands). WC was measured to the nearest 0.1 cm using a tape measure at the level of the umbilicus. BMI was calculated as follows:

$$\text{BMI} = \text{body mass (kg)} / \text{height (m)}^2$$

3.4 Blood pressure

Systolic and diastolic blood pressure were measured from the left arm using an automated monitor (M6 Comfort, Omron, Milton Keynes, UK) and with an appropriately sized cuff. Participants were seated for a minimum of 15 min before measurements and sat quietly (without talking) for at least the final 5 min of this period. A minimum of three measurements were taken, the first of which was excluded, until blood pressure was stable. The mean of stable measurements was calculated.

3.5 Fingertip capillary blood sampling

Fingertip capillary blood sampling (CardioCheck, PTS diagnostics, Indianapolis, USA) was used to measure fasted glucose, TG, total cholesterol and HDL during screening procedures only (*see Section 3.12 for details regarding the measurement of these biomarkers as study outcomes*). Capillary (whole-blood) samples were analysed immediately after collection and LDL was estimated using the Friedewald equation (Friedewald, Levy and Fredrickson, 1972):

$$\text{LDL} = \text{total cholesterol} - \text{HDL} - (\text{TG} / 2.2)$$

3.6 Measurement of expired gases

In Chapters 4, 5 and 6, expired gases were measured continuously during submaximal and maximal exercise testing by indirect calorimetry (Metalyser 3B, Cortex Biophysik GmbH, Germany). Expired gases were also collected during experimental trials in Chapter 4. These gas analysers were serviced annually during the period these studies were undertaken and were calibrated prior to each use. A manual 3 L syringe was used to calibrate gas volume and a two-stage calibration of air composition was performed. The latter involved calibration first against ambient air and then against a bottled cylinder of known gas composition (approximately 17% O_2 and 5% CO_2; supplied by Cranlea Human Performance Ltd., UK). Participants were fitted with an appropriately sized mask (Hans Rudolph 7450 Series, Cranlea Human Performance Ltd., UK), which was kept consistent for each visit. The same gas analyser was used for each participant across multiple visits.

3.7 Submaximal exercise testing

3.7.1 Exercise protocol

During the exercise trial of Chapter 4, participants were required to run for 60 min at an intensity relative to $\dot{V}O_2$ peak (60%). To predict the treadmill speed required to elicit this intensity, a submaximal exercise test was performed. During this test, participants completed four stages of steady-state exercise on a motorised treadmill, each lasting four minutes. All stages were performed at 0% gradient but intensity increased progressively by increasing treadmill speed. Treadmill speeds were chosen in consultation with the participant (considering their habitual activity levels) with the intention of progressing from a light jog during stage one through to a hard, but not exhaustive, run in the final stage.

3.7.2 Determination of treadmill speed required to elicit target relative intensity

Mean steady-state values of oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were calculated for each submaximal exercise stage using data collected during the penultimate 30 s of each stage (i.e. 3:01 to 3:30 min). Any stage in which RER was > 1.0 was excluded. Bivariate regression was performed between treadmill speed and steady state $\dot{V}O_2$ to predict the treadmill speed required to elicit 60 % of $\dot{V}O_2$ peak for use in experimental trials.

3.8 Maximal exercise testing

3.8.1 Exercise protocols

Maximal exercise tests were also performed in Chapters 4, 5 and 6 to assess $\dot{V}O_2$ peak as a marker of cardiorespiratory fitness. In Chapter 6, peak power output (PPO) was also assessed. Tests were performed using the same exercise mode as that utilised within experimental testing of each study. Accordingly, a motorised treadmill was used in Chapters 4 (Excite Med Technogym, Italy) and 5 (Woodway PPS 70 Plus, Woodway Inc., USA), whilst a stationary electromagnetically-braked cycle ergometer (Excalibur Sport, Lode BV, the Netherlands) was used in Chapter 6.

In Chapter 4, participants completed maximal exercise testing using a treadmill speed predicted to elicit a steady-state heart rate (HR) of approximately 150 beats·min^{-1}. This was determined using data from the submaximal exercise test performed approximately 15 min prior (*see Section 3.7*). In Chapter 5, participants were asked to self-select a speed that constituted a 'brisk walk'. In both chapters, participants completed a 3 min warm-up at the selected speed on a minimal gradient (Chapter 4 = 1 %; Chapter 5 = 0 %), after which the test commenced and the gradient was increased by 1 % each min.

In Chapter 6, participants completed a 5 min warm-up at 50 W, after which a ramp protocol was initiated with workload increasing by 16 W·min^{-1}. Participants were instructed to maintain a pedalling cadence of approximately 80 revolutions·min^{-1} (rpm). Participants were familiarised with this test approximately seven days before baseline assessments.

In each of these protocols, HR (T31, Polar Electro (UK) Ltd., United Kingdom) and rating of perceived exertion (RPE) (Borg, 1970) were recorded during the penultimate 10 s of each minute, whilst expired gases were measured throughout (*see Section 3.6*). Tests continued until one of the following occurred:

- Test aborted due to abnormal ECG or other adverse event beyond expected levels of fatigue (this was classified as an incomplete test).
- HR within 10 beats of age-predicted maximum (220 – age) AND respiratory exchange ratio (RER) > 1.15.
- Volitional exhaustion (*all chapters*) OR participants unable to maintain a pedalling cadence of 80 rpm (*Chapter 6 only*).

3.8.2 Determination of peak oxygen uptake ($\dot{V}O_2$ peak) and peak power output (PPO)

Breath-by-breath data were exported at 1 s intervals. $\dot{V}O_2$ peak was considered the maximum $\dot{V}O_2$ value when a rolling 30 s mean was calculated throughout maximal exercise tests. This invariably occurred within the final 2 min of the exercise test. No validation stage was performed in the studies within this thesis. PPO was measured as the power elicited at the point at which the test was stopped (Chapter 6 only).

3.9 Magnetic resonance procedures

In Chapter 6, magnetic resonance spectroscopy and imaging (MRS / MRI) were used for the measurement of IHTG, VAT and ScAT. All MR measurements were performed, after an overnight fast, on a Philips Achieva 3T system using a 32 channel XL-Torso coil, with a total scan duration of approximately 60 minutes.

IHTG was measured from a 20x20x20 mm voxel within the right lobe of the liver using proton-MRS (^{1}H-MRS) with Stimulated Echo Acquisition Mode (STEAM) localization (repetition time=2046 ms to remove T_1 bias) (Bawden, Scott and Aithal, 2017). Water-suppressed and unsuppressed spectra were acquired at four echo times (20, 30, 40 and 60 ms) and used to determine T_2-corrected lipid-to-water ratios (lipid:water). IHTG was quantified as follows (Stephenson *et al.*, 2013):

IHTG = (lipid:water) / [1+(lipid:water)] x 100

VAT and ScAT were measured by MRI using a two-point modified Dixon technique (Philips), acquired in the transverse plane centred in L4/L5 of the spine. A fat mask was generated from fat images using a minimum threshold cut-off in intensity histograms (Nakai *et al.*, 2010) and an in-house algorithm generated fat boundaries of visceral and subcutaneous regions to calculate VAT and ScAT volumes.

3.10 Dual-step hyperinsulinaemic, euglycaemic clamp procedures

In Chapter 6, hepatic and peripheral insulin sensitivity were assessed using a modified version of the hyperinsulinaemic, euglycaemic clamp technique as previously described (DeFronzo, Tobin and Andres, 1979; Johnston *et al.*, 2013). All procedures were performed after an overnight fast.

A dual-stepped protocol was employed with stages of low- (20 $mU \cdot m^{-2} \cdot min^{-1}$) and high-dose (50 $mU \cdot m^{-2} \cdot min^{-1}$) insulin infusion, each lasting 120 minutes. A primed (4 $mg \cdot kg^{-1}$), continuous (0.04 $mg \cdot kg^{-1} \cdot hour^{-1}$) infusion of [6,6-D2] glucose tracer was started 120 minutes before the first hyperinsulinaemic stage and continued throughout to quantify the rate of endogenous glucose production (EGP). Arterialised venous blood glucose was measured every five minutes (YSI 2300 Stat plus, United Kingdom) and euglycaemia (4.5 $mmol \cdot L^{-1}$) was maintained by a variable infusion of 20% dextrose (mean coefficient of variation (CV) ± SD = 1.6 ± 0.9 and 2.7 ± 1.1% at steady-state low- and high-dose insulin infusion, respectively). Each 100g bag of 20% dextrose was spiked with 1g of [6,6-D2] glucose to maintain plasma tracer enrichment. Aliquots of plasma and serum were collected every 15 minutes for subsequent batch analysis of glucose tracer enrichment and insulin concentration, respectively. An example data collection sheet can be found in Appendix VIII.

Plasma glucose isotope enrichment (atoms percent excess) was quantified as the oxime/trimethylsilyl derivative via gas chromatography mass spectrometry (GC-MS; 7890B, MSD 5977A; Agilent Technologies, UK) using selected ion monitoring of the ions at m/z 319 and 321 (CV = 6.4%). Rates of EGP in the basal state and at low-dose insulin infusion were calculated (Wolfe and Chinkes, 2005; Vella and Rizza, 2009) to allow assessment of hepatic insulin sensitivity in the basal and insulin-stimulated states, using the hepatic insulin sensitivity index (HISI) and percentage suppression of EGP during low-dose insulin infusion ($\%EGP_{supp}$), respectively. Full details of glucose tracer calculations can be found in Appendix VIII, whilst HISI and $\%EGP_{supp}$ were calculated as outlined below (*see Section 3.14*).

3.11 Isolation and storage of plasma and serum

Venous blood samples were collected into ice-cooled tubes (Chapters 4 and 5: Monovette, Sarstedt, Leicester, UK; Chapter 6: Vacutainer, BD and Co., NJ, USA) which, apart from two exceptions (outlined below), were pre-treated with anticoagulant for the isolation of plasma. EDTA or Lithium Heparin were used as anticoagulant, dependent on analytical instructions. After collection, tubes were gently inverted 7-10 times and spun immediately in a refrigerated centrifuge (4 ^{o}C) for ≥10 min at 2383 x *g*. The plasma supernatant was removed and aliquoted for storage at -80 ^{o}C. During hyperinsulinaemic, euglycaemic clamps (Chapter 6), samples for the measurement of serum insulin were collected into tubes pre-treated with a clotting factor and left for 30 min prior to centrifugation. Plasma samples collected for the measurement of glucose tracer enrichment were pre-treated with fluoride and heparin.

3.12 Biochemical analyses

Commercially available enzyme-linked immunosorbent assays (ELISAs) were used to measure plasma concentrations of FGF21, follistatin, fetuin-A (R & D Systems, Oxford, UK), LECT2 (MBL International, Massachusetts, USA), insulin (Chapters 4 and 5 only) and glucagon (Chapter 4 only) (Mercodia, Uppsala, Sweden). All assays were performed according to manufacturer instructions, including respective curve fitting, with dilutions and washes performed manually using an automated multi-channel pipette (Sartorius, NY, USA). Measurement of absorbance and subsequent curve fitting were performed on an automated plate reader (Varioskan Flash Multiple Mode Reader, Thermo Scientific, Vantaa, Finland). In Chapter 6, serum insulin was quantified using radioimmunoassay (Millipore, USA). Plasma concentrations of full-length SeP were measured using a sol particle homogeneous immunoassay, utilising two types of SeP monoclonal antibody, as previously reported (Saito *et al.*, 2001; Tanaka *et al.*, 2016). In Chapters 4 and 6, circulating concentrations of glucose, TG, NEFA, total cholesterol, HDL, AST, ALT and GGT were analysed by enzymatic colorimetric methods using a benchtop analyser (Pentra 400; HORIBA ABX Diagnostics, Montpellier, France). In Chapter 5, plasma glucose, TG, total cholesterol, HDL and HbA1c were measured by the pathology laboratories of the University Hospitals of Leicester NHS Trust, whilst NEFA was sent to specialist laboratories at Nottingham University Hospitals NHS Trust.

3.13 Calculation of plasma volume and adjustment of plasma protein concentrations

In Chapter 4, where samples were collected on multiple occasions throughout trial days, blood haematocrit and haemoglobin concentrations were determined in each sample to monitor changes in plasma volume, calculated using established equations (Dill and Costill, 1974). Haematocrit was measured in triplicate with the collection of whole blood into heparinised microtubes, which were subsequently spun in a microliter-haematocrit centrifuge (MIKRO 20, Andreas Hettich GmbH and Co., Tuttlingen, Germany). Haemoglobin was measured in duplicate by the cyanmethaemoglobin method using a spectrophotometer (CECIL CE1011, Cecil Instruments Ltd., Cambridge, UK) from samples collected into a micropipette and dispensed into Drabkin's solution. Circulating protein concentrations were corrected, as previously described (Sherk *et al.*, 2013), when plasma volume deviated significantly from baseline. In Chapter 5, haematocrit and haemoglobin were requested from the pathology

laboratories of the University Hospitals of Leicester NHS Trust but, due to large amounts of missing data (~33%), adjustments for changes in plasma volume were not performed.

3.14 Calculation of indices of insulin resistance

Throughout this thesis, HOMA-IR (Matthews *et al.*, 1985), adipose tissue insulin resistance index (Adipo-IR) (Gastaldelli *et al.*, 2007) and HISI (Matsuda and DeFronzo, 1999) were calculated as follows:

HOMA-IR = fasted plasma glucose [$mmol \cdot L^{-1}$] x fasted plasma insulin [$mU \cdot L^{-1}$] / 22.5

Adipo-IR = fasted plasma NEFA [$mmol \cdot L^{-1}$] x fasted plasma insulin [$pmol \cdot L^{-1}$]

HISI = 1000 / basal EGP [$mg \cdot m^{-2} \cdot min^{-1}$] x fasted plasma insulin [$mU \cdot L^{-1}$]

In Chapters 6 and 7, hepatic insulin sensitivity was also assessed in the insulin-stimulated state using the percentage suppression of EGP during low-dose insulin infusion ($\%EGP_{supp}$), calculated as follows:

$\%EGP_{supp}$ = (EGP at low-dose insulin infusion – basal EGP) / basal EGP x 100

3.15 Calculation of area under the curve (AUC)

In Chapters 4 and 5, the total area under the concentration-time curve (AUC) for circulating biomarkers in each experimental trial was calculated using the trapezoid method. Total AUC was calculated as the sum of the areas between each consecutive measurement:

Area between measurement A and B = $(T_B - T_A) \times (0.5 \times [C_A + C_B])$

where T = time and C = concentration.

3.16 Statistical analyses

Statistical analyses were performed using commercially available software (Chapters 4, 5 and 6: SPSS version 23.0, SPSS Inc., USA; Chapter 7: Stata IC, Version 14.1, StataCorp LP, Texas, USA). In Chapters 4, 5 and 6, data were first assessed for their suitability for parametric statistical testing. Specifically, Shapiro-Wilk tests were performed to assess the distribution of data and in Chapter 4, which contained a between-subjects factor, Levene's test was used to assess homogeneity of variance between groups. In bivariate correlation analyses, the distribution of each individual variable and the standardised residuals were assessed. If either the normality of distribution or homogeneity of variance assumptions were violated, a natural logarithmic transformation was applied to the raw data and the parametric assumptions were

re-assessed. If the assumptions were still not met, non-parametric statistical tests were used. All experimental studies in this thesis involved at least one within-measures factor and thus, when parametric tests were performed, Mauchly's test of sphericity was used to determine whether the variances between all the pairs of measurements were similar. When sphericity could not be assumed, a correction was applied to the degrees of freedom. In these instances, the Greenhouse-Geisser epsilon was consulted and when < 0.75, this correction was used. When the Greenhouse-Geisser epsilon was > 0.75 the Huynh-Feldt correction was applied (Atkinson, 2001).

All normally distributed data are presented as the arithmetic mean with either standard deviation or standard error of mean (Lydersen, 2015). Non-normally distributed data are presented as the median with interquartile range (IQR). Statistical significance was considered at the 5% level ($P \leq 0.05$) and, where appropriate, probability (P-) values were adjusted for multiple comparisons using the Holm-Bonferroni correction (Holm, 1979), to reduce the chance of type I statistical error.

CHAPTER 4

THE INFLUENCE OF ADIPOSITY AND ACUTE EXERCISE ON CIRCULATING HEPATOKINES IN NORMAL WEIGHT AND OVERWEIGHT/OBESE MEN

The study presented in this chapter has been published and has the following citation:

> Sargeant JA, Aithal GP, Takamura T *et al*. (2018). *Applied Physiology Nutrition and Metabolism.* **43**: 482-490**.**

This manuscript was also selected by the editor as the featured content for the May issue of *Applied Physiology, Nutrition and Metabolism* (Appendix I).

This chapter presents the published manuscript but with some aspects of the methods section condensed or removed to avoid repetition within this thesis. When this occurs, readers are directed to the appropriate sections of the General Methods for more information.

4.1 Abstract

Background: Hepatokines are liver-secreted proteins with potential to influence glucose regulation and other metabolic parameters. This study investigated differences in adiposity status on five novel hepatokines and characterised their response to acute moderate-intensity exercise in groups of normal weight and overweight/obese men.

Methods: Twenty-two men were recruited into normal weight and overweight/obese groups (BMI: 18.5 to 24.9 and 25.0 to 34.9 $kg \cdot m^{-2}$). Each completed two experimental trials, exercise and control. During exercise trials, participants performed 60 min of moderate-intensity treadmill exercise (~60% $\dot{V}O_2$ peak) and then rested for 6 h. Participants rested throughout control trials. Circulating fibroblast growth factor-21 (FGF21), follistatin, leukocyte cell-derived chemotaxin 2 (LECT2), fetuin-A and selenoprotein-P (SeP) were measured throughout.

Results: Fasted (resting) FGF21 and LECT2 were higher in overweight/obese individuals (129% and 55%; $P \leq 0.01$) and correlated with indices of adiposity and insulin resistance; whereas circulating follistatin was lower in overweight/obese individuals throughout trial days (17%, $P < 0.05$). In both groups, circulating concentrations of FGF21 and follistatin were transiently elevated after exercise for up to 6 h ($P \leq 0.02$). Circulating fetuin-A and SeP were no different between groups ($P \geq 0.19$) and, along with LECT2, were unaffected by exercise ($P \geq 0.06$).

Conclusions: These findings show that increased adiposity is associated with a modified hepatokine profile, which may represent a novel mechanism linking excess adiposity to metabolic health. Furthermore, acute perturbations in circulating FGF21 and follistatin after exercise may contribute to the health benefits of an active lifestyle.

4.2 Introduction

Recent work characterising the hepatic proteome has identified 168 proteins which can be secreted and potentially exert endocrine-like effects in distal sites (Meex *et al.*, 2015). A number of these 'hepatokines' are associated with measures of adiposity (Chen *et al.*, 2011; Xu *et al.*, 2011; Yang, Hwang, *et al.*, 2011; Lan *et al.*, 2014) and have been shown to exert metabolic effects within various central and peripheral tissues (Misu *et al.*, 2010; Camporez *et al.*, 2013; Malin, Mulya, *et al.*, 2013; Lan *et al.*, 2014; Hansen, Rutti, *et al.*, 2016). Together, this evidence has prompted suggestions that hepatokines represent a potential mechanism linking adiposity and metabolic health and may be novel therapeutic targets to combat obesity-related insulin resistance and associated metabolic disease.

To date, much of the research concerning hepatokine function and metabolism has focused on their direct influence on tissue-specific insulin sensitivity and systemic glucose metabolism. The most frequently studied, fibroblast growth factor-21 (FGF21), has been shown to improve glucose metabolism in the liver, skeletal muscle and adipose tissue (Camporez *et al.*, 2013); whilst follistatin may promote pancreatic beta cell survival and suppress circulating glucagon (Hansen, Rutti, *et al.*, 2016). Other hepatokines may act to promote insulin resistance. For example, within skeletal muscle, leukocyte cell-derived chemotaxin 2 (LECT2) (Lan *et al.*, 2014), selenoprotein-P (SeP) (Misu *et al.*, 2010) and fetuin-A (Malin, Mulya, *et al.*, 2013) have each been shown to directly interfere with distinct aspects of glucose metabolism. Observational evidence in humans has identified associations between these hepatokines and adiposity, insulin resistance, ectopic lipid and the metabolic syndrome (Zhang *et al.*, 2008; Li *et al.*, 2010; Chen *et al.*, 2011; Xu *et al.*, 2011; Yang, Hwang, *et al.*, 2011; H. Y. Choi *et al.*, 2013; Hansen *et al.*, 2013; Okumura *et al.*, 2013; Lan *et al.*, 2014). However, human experimental research is now required to scrutinise the pathophysiological relevance of these novel proteins *in vivo*.

Current evidence demonstrates that exercise training reduces circulating levels of fetuin-A and FGF21, and responses correlate with improvements in insulin sensitivity and intrahepatic fat (Malin, Mulya, *et al.*, 2013; Malin *et al.*, 2014; Taniguchi *et al.*, 2016). Given that single bouts of exercise transiently enhance insulin sensitivity (Sylow *et al.*, 2017), a handful of studies have also investigated the acute influence of exercise on circulating hepatokines, speculating that modulation of the hepatokine profile may be implicated in the benefits induced. This hypothesis is strengthened by the knowledge that exercise acutely increases circulating non-

esterified fatty acids (NEFA) and glucagon, and activates hepatic AMP-activated protein kinase (AMPK) (Camacho *et al.*, 2006; Hansen, Pedersen, *et al.*, 2016). Each of these have been implicated in the regulation of at least one of the hepatokines outlined above (Jung, Choi, *et al.*, 2013; Lan *et al.*, 2014; Trepanowski, Mey and Varady, 2014; Hansen, Pedersen, *et al.*, 2016). Whilst these studies remain limited in number, the available evidence shows that moderate- to high-intensity aerobic exercise acutely increases circulating levels of FGF21 and follistatin (Slusher *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016), but responses may differ between normal weight and obese individuals, and between individuals with normal and dysregulated glucose metabolism (Slusher *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016). Additional work is required to determine whether the FGF21 and follistatin responses to acute exercise differ between normal weight and overweight/obese individuals who are free of diagnosed metabolic disease, and whether similar responses occur in other relevant hepatokines.

The purpose of this study was two-fold. Firstly, we sought to investigate differences in adiposity status on FGF21, follistatin, LECT2, SeP and fetuin-A in normal weight and overweight/obese men. Secondly, we characterised the effect of an acute bout of moderate-intensity exercise on circulating concentrations of these hepatokines in order to explore their potential role as mediators of exercise-induced improvements in glycaemic control and other metabolic parameters. We hypothesised that overweight/obese individuals would have elevated circulating concentrations of hepatokines at rest and that an acute bout of moderate-intensity exercise would beneficially alter circulating hepatokine profiles; by reducing LECT2, SeP, and fetuin A, whilst increasing FGF21 and follistatin.

4.3 Materials and methods

4.3.1 Ethical approval and participant recruitment

After receiving approval from the East Midlands NHS Research Ethics committee (13/EM/0290), 22 non-smoking men were recruited equally into normal weight and overweight/obese groups (BMI: 18.5 to 24.9 and 25.0 to 34.9 $kg \cdot m^{-2}$, respectively); providing written, informed consent to participate (*see Section 3.1 for more details*). This sample size was chosen based on previous studies that documented significant changes in hepatokines (FGF21 and follistatin) in response to acute exercise; as well as differences between participant groups (normal weight vs. obese and normal vs. impaired glucose regulation) (Slusher *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016). Participants in the current study were free of diagnosed chronic disease and were not taking medications known to affect glucose or lipid metabolism, or blood pressure. Participants were also 'inactive' or 'moderately active' according to the IPAQ (www.ipaq.ki.se [accessed 03/01/2017]) and were weight stable in the three months prior to enrolment (< 2 kg self-reported weight change).

4.3.2 Participant pre-assessment

During a pre-assessment visit, participants were screened to determine eligibility and suitability for exercise testing as outlined in General Methods (*see Section 3.2*). Height, weight, BF % and WC were also measured (*see Section 3.3*). In this study, participants were excluded if blood pressure was greater than 160/100 mmHg.

On a separate occasion, participants completed a 16-minute, progressive sub-maximal exercise test followed, after approximately 15 minutes, by a ramped maximal exercise test to determine $\dot{V}O_2$ peak and predict the treadmill speed to elicit 60 % of $\dot{V}O_2$ peak during experimental trials (*see Sections 3.6 to 3.8 of General Methods for further detail*).

In the 48 h before main trials, participants refrained from strenuous physical activity, alcohol and caffeine, and standardised their dietary intake using weighed records. On the evening before main trials, participants were provided with a standardised meal (3138 kJ; 71% carbohydrate, 18% fat, 11% protein) to consume before 21:00, after which only water was permitted until the start of the trials.

4.3.3 Experimental trials

Participants completed two experimental trials, control and exercise, in a counterbalanced order and separated by approximately one week. On the morning of each trial, participants arrived at the laboratory at 08:30, at which point an intravenous cannula (21 G; Venflon, Becton Dickinson, Sweden) was inserted into an antecubital vein. Trials were then initiated with a venous blood sample taken at ~09:00 (0 h) and additional samples were collected at 1, 1.5, 2.75, 4 and 7 h (Figure 4.1). During exercise trials, participants completed a 60-min bout of moderate-intensity treadmill exercise (60% of $\dot{V}O_2$ peak) between 0 and 1 h, and then rested in the laboratory for the remainder of the trial (1-7 h). HR and RPE (Borg, 1970) were recorded every 15 min during exercise, and expired air was collected throughout for ongoing measurement of $\dot{V}O_2$ and $\dot{V}CO_2$. If necessary, treadmill speed was adjusted to maintain the desired exercise intensity. Participants rested for the entirety of control trials and samples of expired air were collected between 0 and 1 h to quantify resting energy expenditure; allowing the calculation of net energy expenditure elicited by exercise. Participants were provided with a standardised breakfast (2690 kJ; 72% carbohydrate, 18% fat, 10% protein) and lunch (3138 kJ; 43% carbohydrate, 25% fat, 32% protein) at 1.5 and 4 h, respectively.

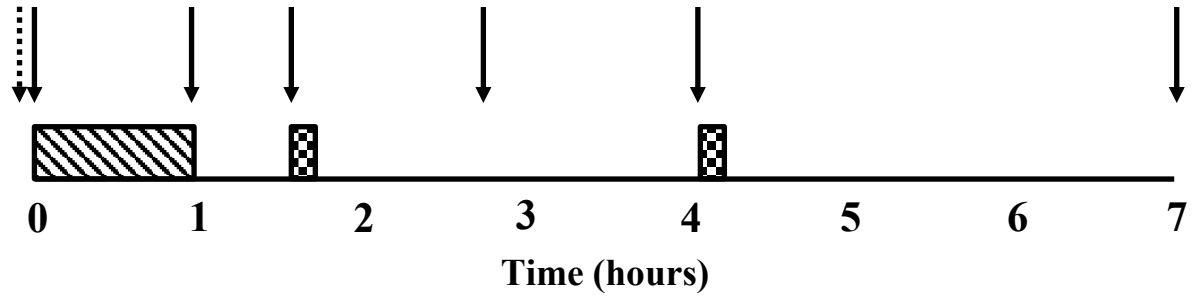


Figure 4.1 Schematic representation of experimental trial days.

Acute moderate-intensity exercise (exercise trials) or measurement of resting energy expenditure (control trials)

Standardised meal consumed

Venous cannula inserted

Venous blood sample collected

4.3.4 Biochemical analyses

Venous blood samples were collected into ice-cooled monovettes pre-treated with anticoagulant (Sarstedt, Leicester, UK) and plasma was isolated as outlined in General Methods (*see Section 3.11*). Plasma concentrations of FGF21, follistatin, fetuin-A, LECT2, insulin, glucagon and SeP were measured at each time point (*see Section 3.12)*. The mean within-batch co-efficient of variation (CV) for these assays was ≤ 5.5%. Circulating concentrations of NEFA, glucose, TG, total cholesterol, AST, ALT and GGT were also measured (within batch CV ≤ 4.2%). All were measured at 0 h only with the exception of plasma NEFA, which was measured at each sample time point. Insulin resistance was assessed by HOMA-IR and Adipo-IR (*see Section 3.14*) (Matthews *et al.*, 1985; Gastaldelli *et al.*, 2007).

Plasma volume was calculated in each whole blood sample using established equations (Dill and Costill, 1974) and circulating protein concentrations were corrected when plasma volume deviated significantly from baseline (*see Section 3.13* for more details).

4.3.5 Statistical analyses

Two-tailed, independent samples *t*-tests were used to compare differences in participant characteristics, fasted plasma protein and metabolite concentrations, and characteristics of the exercise performed between normal weight and overweight/obese groups. When parametric assumptions were not met before or after log transformation, non-parametric Wilcoxon matched-pairs signed rank test was used. Relationships between fasted hepatokine concentrations and other participant characteristics were assessed using bivariate Pearson's and Spearman's correlation analyses as appropriate. Three-way, mixed-design analysis of variance (ANOVA), consisting of two within-participant factors (trial and sample time) and one between-participant factor (group), was used to assess hepatokine responses to exercise. After inspection of main effects, the three-way interaction between trial, time and group was used to assess whether hepatokine responses during and after exercise, when compared to the control trial, differed between normal weight and overweight/obese groups. When this was not significant, the two-way interaction between trial and time was used to investigate the hepatokine response to exercise in the two groups combined. Statistically significant two-way interactions were investigated further with two-tailed paired samples *t*-tests to identify the times at which circulating concentrations differed between control and exercise trials. Due to the sample size in this study, no correction for multiple comparisons was applied. To help clarity in graphical presentation, total area under the concentration-time curve (AUC) was also

calculated for each experimental trial (*see Section 3.15*) and these data were analysed statistically using two-way mixed design ANOVA.

4.4 Results

4.4.1 Participant characteristics

Descriptive characteristics of the normal weight and overweight/obese groups can be found in Table 4.1. By design, the overweight/obese group had higher BMI, body mass, body fat percentage and waist circumference, but age was similar between groups. There was no difference in absolute cardiorespiratory fitness between groups but relative fitness was greater in the normal weight individuals due to their lower body mass. Fasted plasma glucose, insulin and HOMA-IR were similar between groups, but fasted plasma lipids were greater in the overweight/obese individuals, whilst Adipo-IR tended to be higher. There were no significant differences in AST, ALT or GGT between groups (all $P \geq 0.35$; data not shown).

4.4.2 Fasted plasma hepatokine concentrations and associations with metabolic variables

The overweight/obese individuals had greater fasted plasma concentrations of LECT2 and FGF21, but fasted concentrations of follistatin, fetuin-A and SeP were similar between groups (Table 4.1). Fasted circulating LECT2 and FGF21 were positively correlated with each other ($rho^2 = 36.9\%$, $P = 0.03$), body mass, BMI, WC, BF%, NEFA, TG, Adipo-IR and glucagon ($r^2 \geq 19.4\%$, $P \leq 0.02$ or $rho^2 \geq 17.6\%$, $P \leq 0.05$), and negatively with relative $\dot{V}O_2$ peak ($r^2 \geq 27.0\%$, $P \leq 0.01$). FGF21 was also marginally positively correlated with fasted plasma glucose ($r^2 = 18.1\%$, $P = 0.048$), whilst LECT2 was strongly positively correlated with HOMA-IR ($rho^2 = 43.2\%$, $P = 0.001$). Significant negative correlations were found between fasted concentrations of follistatin and the AST:ALT ratio ($r^2 = 18.5\%$, $P = 0.05$), fetuin-A and age ($rho^2 = 25\%$, $P = 0.02$), and between fasted concentrations of SeP and ALT ($r^2 = 19.4\%$, $P = 0.04$). Further details of all significant correlations can be found in Appendix VII.

Table 4.1 Participant characteristics

	Normal weight (n=11)	Overweight/obese (n=11)
Anthropometry		
BMI ($kg \cdot m^{-2}$) [a]	23.4 (1.6)	29.2 (4.5)‡
Age (years)	36 ± 15	45 ± 14
Body weight (kg)	69.8 ± 1.5	92.3 ± 3.4‡
Body fat (%)	16.9 ± 3.6	26.4 ± 4.0‡
Waist circumference (cm)	81.6 ± 5.3	96.0 ± 7.8‡
Cardiorespiratory Fitness		
Absolute $\dot{V}O_2$ peak ($L \cdot min^{-1}$)	3.46 ± 0.74	3.21 ± 1.21
Relative $\dot{V}O_2$ peak ($mL \cdot kg\ BW^{-1} \cdot min^{-1}$)	50.1 ± 11.9	38.5 ± 9.7†
Circulating Metabolic Risk Factors		
Total cholesterol ($mmol \cdot L^{-1}$)	4.12 ± 0.73	4.91 ± 0.89*
TG ($mmol \cdot L^{-1}$)	1.04 ± 0.15	1.82 ± 0.24†
NEFA ($mmol \cdot L^{-1}$)	0.39 ± 0.18	0.58 ± 0.14†
FPG ($mmol \cdot L^{-1}$)	4.9 ± 0.2	5.0 ± 0.3
FPI ($pmol \cdot L^{-1}$)	30.0 ± 12.2	37.1 ± 19.3
Insulin Sensitivity		
HOMA-IR	0.95 ± 0.39	1.21 ± 0.67
Adipo-IR	12.57 ± 8.99	21.98 ± 13.01
Hepatokines		
FGF21 ($pg \cdot mL^{-1}$)	83 ± 55	190 ± 74‡
Follistatin ($pg \cdot mL^{-1}$)	795 ± 257	670 ± 154
LECT2 ($ng \cdot mL^{-1}$)	31 ± 10	48 ± 17†
Fetuin-A ($\mu g \cdot mL^{-1}$)	541 ± 137	497 ± 99
SeP ($\mu g \cdot mL^{-1}$)	3.01 ± 0.39	2.81 ± 0.30

[a] Heterogeneous variance between groups, non-parametric analyses performed and data presented as median (interquartile range). All other data presented as mean ± SD. Symbols indicate statistically significant differences between groups (* < 0.05; † ≤ 0.01; ‡ < 0.001).

4.4.3 Exercise characteristics

Participants in the normal weight group exercised at a greater treadmill speed due to their higher relative cardiorespiratory fitness. However, the relative intensity of the exercise performed was similar between groups (Table 4.2). Consequently, given the higher energy cost of exercise in the overweight/obese group as a result of their higher body mass, the net energy expenditure during exercise trials was similar between groups ($P = 0.98$). During the exercise trials, there was a significant reduction in plasma volume immediately post-exercise irrespective of group (0 vs 1 h: 58.5 ± 0.7 vs. 53.7 ± 0.7%; $P < 0.01$), which returned to baseline by 1.5 h.

Table 4.2 Exercise characteristics

	Normal weight (n=11)	**Overweight/obese (n=11)**
Treadmill speed ($km \cdot h^{-1}$)	7.6 ± 1.0	6.8 ± 1.0*
$\dot{V}O_2$ elicited (%$\dot{V}O_2$ peak)	59.3 ± 2.8	57.9 ± 2.3
Net energy expenditure (kJ)	2211 ± 507	2217 ± 509
Heart rate ($beats \cdot min^{-1}$)	141 ± 29	139 ± 19
Rating of perceived exertion (6-20)	13 ± 1	12 ± 1

Data presented as mean ± SD. *indicates a statistically significant difference between groups ($P < 0.05$).

4.4.4 Circulating hepatokine responses to exercise

Plasma FGF21 concentrations were higher in the overweight/obese group, irrespective of trial or time (Figure 4.2a; $P = 0.003$), but there was no interaction between trial, time and group ($P = 0.19$). With groups combined, there was a significant two-way interaction between trial and time for circulating FGF21 (Figure 4.3a; $P < 0.001$), and *post-hoc* analyses revealed that circulating concentrations were significantly higher at 1, 1.5 and 4 h in the exercise trial, compared with control (all $P \leq 0.005$). Accordingly, the total AUC for FGF21 was significantly greater in the overweight/obese compared with the normal weight individuals (Figure 4.2b; $P = 0.003$), and in the exercise versus the control trials (Figure 4.3b; $P = 0.003$). However, there was no interaction between group and trial ($P = 0.65$).

Circulating follistatin was lower in the overweight/obese versus the normal weight group (Figure 4.2c; $P = 0.05$), but the interaction between trial, time and group was not significant ($P = 0.94$). In the whole study population, there was a significant two-way interaction between trial and time for plasma follistatin (Figure 4.3c; $P = 0.001$). *Post-hoc* analyses identified significantly higher concentrations at 2.75, 4 and 7 h in the exercise trial ($P \leq 0.02$). Similarly, the total AUC for follistatin was significantly lower in the overweight/obese group (Figure 4.2d; $P = 0.05$) and greater in the exercise trials (Figure 4.3d; $P < 0.01$), but there was no interaction between group and trial ($P = 0.41$).

Circulating LECT2 was higher in the overweight/obese group versus the normal weight group, (Figure 4.2e; $P = 0.009$), but there was no interaction between trial, time and group ($P = 0.38$). For plasma concentrations of LECT2, the two-way interaction between trial and time in the whole study population was also not significant (Figure 4.3e; $P = 0.06$). The total AUC analyses for LECT2 mirrored these results with a significantly greater AUC in the overweight/obese group (Figure 4.2f; $P < 0.01$), but no significant difference between the control and exercise trials (Figure 4.3f; $P = 0.07$) and no interaction between group and trial ($P = 0.45$).

Circulating concentrations of fetuin-A and SeP were similar between groups (Figures 4.2g and 4.2i; $P \geq 0.20$) and there were no interactions between trial, time and group ($P \geq 0.07$). Furthermore, with groups combined, there were no interactions between trial and time (Figures 4.3g and 4.3i; $P \geq 0.11$). In accordance, the total AUC for fetuin-A (Figures 4.2h and 4.3h) and SeP (Figures 4.2j and 4.3j) were similar between groups and between trials, and there were no significant interactions (all $P \geq 0.17$).

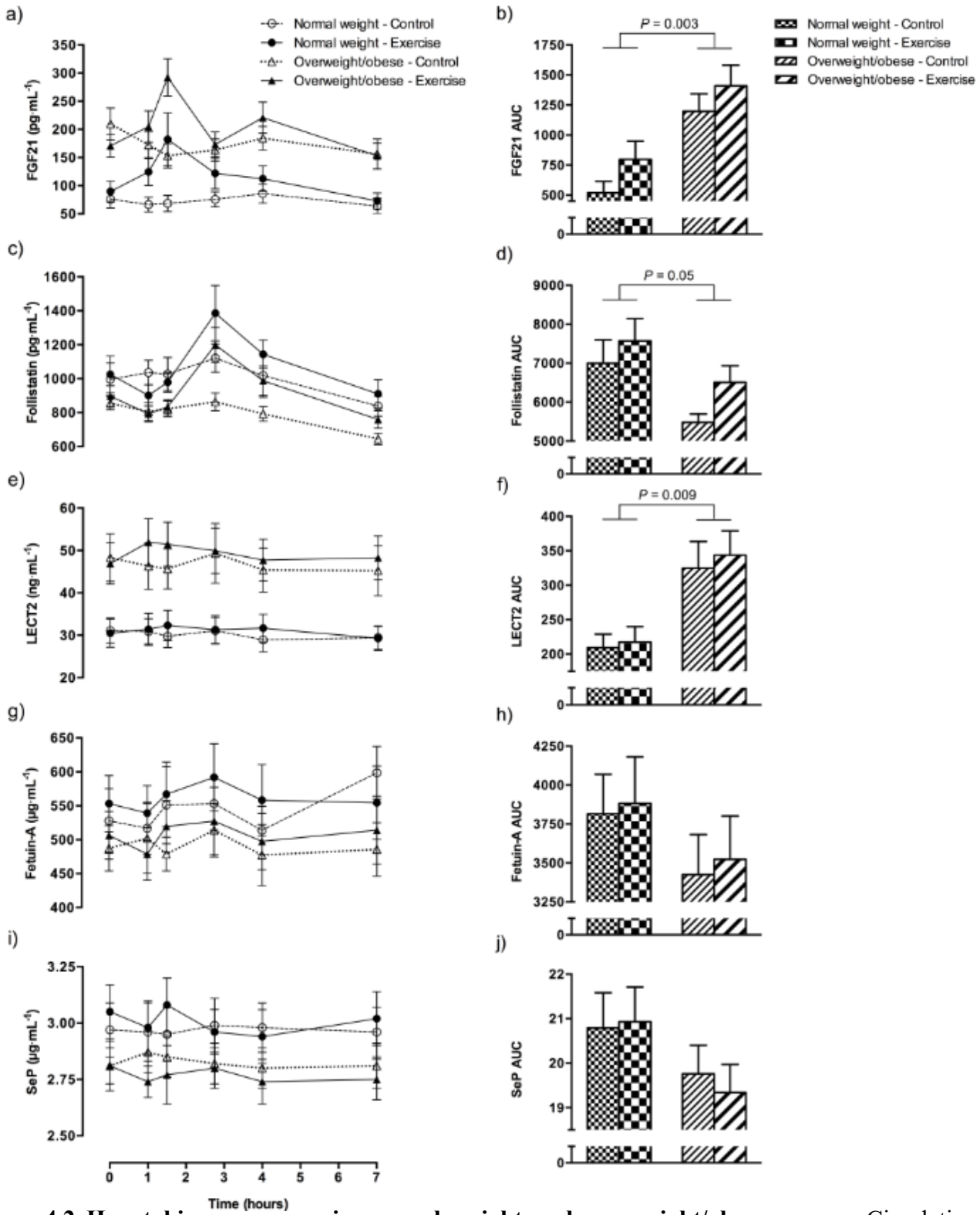


Figure 4.2 Hepatokine responses in normal weight and overweight/obese groups. Circulating plasma concentrations FGF21 (a-b), follistatin (c-d), LECT2 (e-f), fetuin-A (g-h) and SeP (i-j) during control and exercise trials in both normal weight and overweight/obese groups. Meals were provided at 1.5 and 4 h. Exercise was performed between 0 and 1 h in the exercise trial only. Data presented as mean ± SEM. AUC represents the total area under the concentration-time curve for the given experimental day. *P*-values denote significant main effect of group irrespective or time or trial.

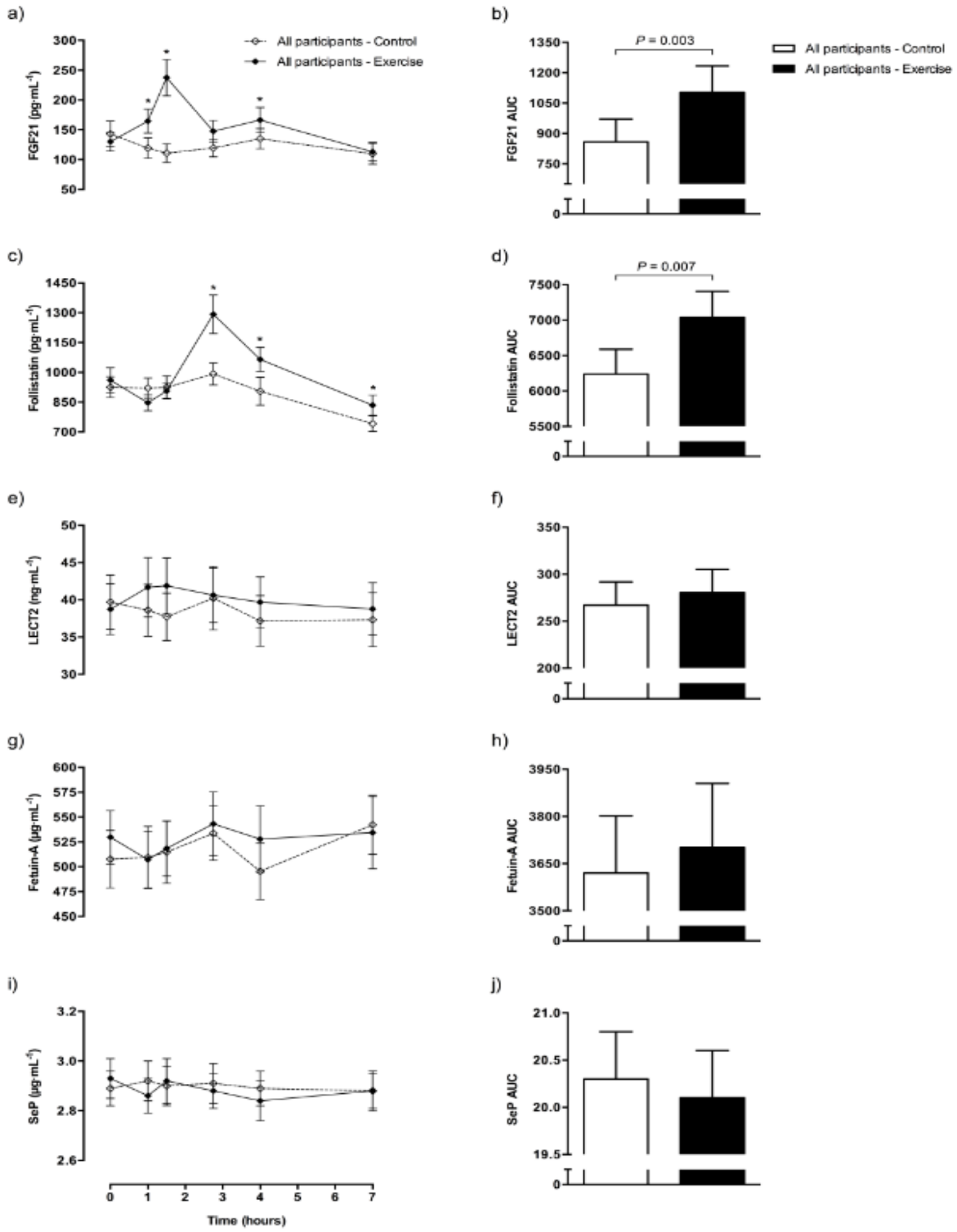


Figure 4.3 Hepatokine exercise responses with groups combined to a single population. Circulating plasma concentrations FGF21 (a-b), follistatin (c-d), LECT2 (e-f), fetuin-A (g-h) and SeP (i-j) during control and exercise trials in the whole study population combined. Meals were provided at 1.5 and 4 h. Exercise was performed between 0 and 1 h in the exercise trial only. Data presented as mean ± SEM. AUC represents the total area under the concentration-time curve for the given experimental day. * indicates significant difference from control trial at the same time point (all $P \leq 0.02$). *P*-values on AUC plots denote significant difference between control and exercise trials.

4.4.5 Circulating responses of NEFA, glucagon and insulin to exercise

Despite differences in the fasted state, circulating concentrations of NEFA were similar between groups throughout experimental trials (P = 0.09), and there was no significant interaction between trial, time and group (P = 0.13). However, with groups combined there was a significant two-way interaction between trial and time (Figure 4.4a; $P < 0.001$) and *post-hoc* analyses revealed significantly higher concentrations of NEFA in the exercise trial at 1, 1.5, 2.75 and 7 h ($P \leq 0.04$).

Circulating concentrations of glucagon, insulin and glucagon to insulin ratio were also similar between groups ($P \geq 0.27$), and the three-way interactions between trial, time and group were not significant for any of these outcomes ($P \geq 0.16$). However, in the whole study population, there were significant two-way interactions between trial and time for all of glucagon, insulin and the glucagon to insulin ratio (Figure 4.4b-d; $P \leq 0.03$). *Post-hoc* tests revealed the glucagon to insulin ratio was significantly greater at 1 and 1.5 h in the exercise trial when compared to the control trial ($P \leq 0.02$). This was primarily driven by significantly higher concentrations of glucagon ($P \leq 0.01$) and occurred despite significantly higher concentrations of insulin at 1.5 h in the exercise trial (P = 0.02). Glucagon remained elevated in the exercise trial at 2.75 h (P = 0.03) but the consequential increase in the glucagon to insulin ratio was not statistically significant (P = 0.08).

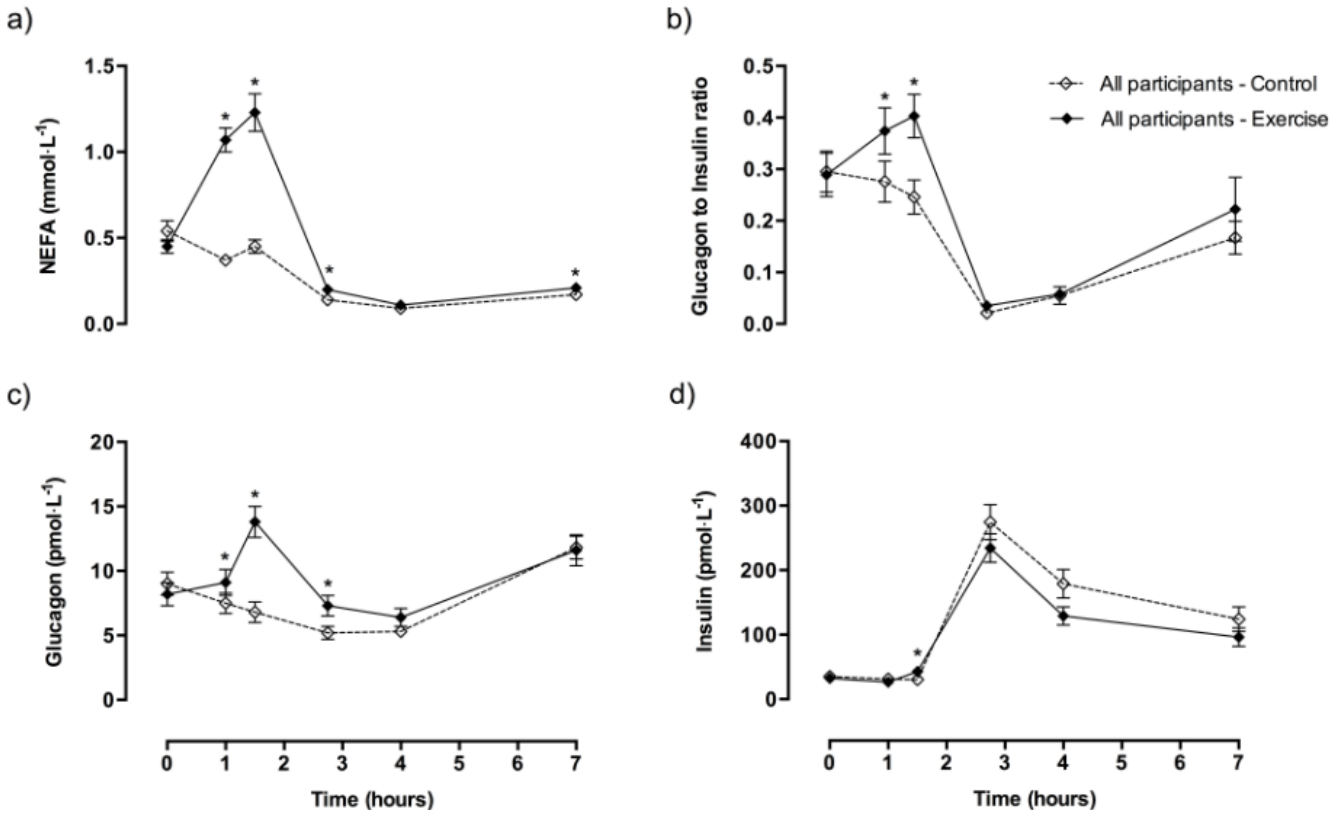


Figure 4.4 Responses in NEFA, glucagon and insulin with groups combined to a single population. Circulating plasma concentrations (a) NEFA (b) glucagon (c) insulin and (d) the glucagon to insulin ratio during control and exercise trials in the whole study population combined. Meals were provided at 1.5 and 4 h. Exercise was performed between 0 and 1 h in the exercise trial only. Data presented as mean ± SEM. * indicates significant difference from control trial at the same time point (all $P \leq 0.04$).

4.5 Discussion

This study investigated the impact of adiposity and acute exercise on five candidate hepatokines which have been identified as novel circulating proteins linking the liver and peripheral metabolism. Our findings suggest that circulating levels of LECT2, FGF21 and follistatin are modulated by adiposity and are associated with various anthropometric measurements and biomarkers of metabolic health. Additionally, our findings show that circulating levels of FGF21 and follistatin are transiently elevated after a single bout of moderate-intensity exercise, and these responses are preserved in overweight/obese individuals. These responses may help mediate the favourable metabolic impact of exercise, but further research is needed to assess causality.

Previous reports have shown increased LECT2 in obese individuals, with or without non-alcoholic fatty liver disease (NAFLD), in two large Japanese cohorts (Okumura *et al.*, 2013; Lan *et al.*, 2014). The current study is the first, however, to show that LECT2 is elevated in European men that are overweight/obese and correlates with BMI in a population of normal weight, overweight and obese individuals. In mice, hepatic expression and circulating concentrations of LECT2 are negatively regulated by hepatic AMPK (Lan *et al.*, 2014). Furthermore, eight weeks of high-fat overfeeding increased circulating concentrations of LECT2, alongside increases in body mass (Lan *et al.*, 2014). As such, circulating LECT2 may be increased in overweight and obese individuals due to chronic reduction of hepatic AMPK activity resulting from sustained energy surplus. In agreement with previous studies (Okumura *et al.*, 2013; Lan *et al.*, 2014), we show significant associations between LECT2 and fasted plasma insulin and HOMA-IR, whilst we also report, for the first time, significant correlations between fasted concentrations of LECT2, NEFA and Adipo-IR. LECT2 has been shown to inhibit insulin signalling in C2C12 myotubes via activation of cJNK (Lan *et al.*, 2014) but its effects on other peripheral tissues, including hepatic and adipose tissues, warrant further investigation.

Previous reports have shown that fasted concentrations of FGF21 are elevated in obese individuals with normal or dysregulated glucose metabolism (Zhang *et al.*, 2008; Chen *et al.*, 2011). Our findings are in agreement with these studies and show that FGF21 is also increased in individuals that are overweight/obese. Given the beneficial metabolic effects associated with FGF21, it may seem somewhat paradoxical that circulating concentrations are increased in individuals with overweight or obesity. However, it has been suggested that increased FGF21

may be part of an early adaptive response to chronic overnutrition, to aid subcutaneous adipose tissue expansion and thus attenuate lipid storage in ectopic sites (Li *et al*., 2018). This may explain why transient increases in circulating FGF21 to acute stimuli, such as exercise, may be apparent and considered beneficial, whilst concentrations are also elevated chronically in individuals with metabolic dysregulation. Hepatic expression and secretion of FGF21 is increased during periods of starvation via the activation of peroxisome proliferator-activated receptor alpha (PPAR-α) by circulating NEFA (Badman *et al.*, 2007). However, plasma concentrations of NEFA are also increased with obesity (Boden, 2008), offering a potential mechanism to explain the increased FGF21 concentrations seen in the present and previous studies. In support of this, we report elevated fasted concentrations of NEFA in the overweight/obese group and a strong positive correlation between circulating concentrations of FGF21 and NEFA. Alternatively, a state of 'FGF21 resistance' may also result in elevated concentrations of FGF21 group (Potthoff, Kliewer and Mangelsdorf, 2012) but it was beyond the scope of this study to investigate this hypothesis.

Despite no statistically significant difference in the fasted state, we showed lower concentrations of follistatin in the overweight/obese group throughout trial days. Our findings are consistent with previous data which identified lower follistatin levels in obese individuals with T2DM (Ueland *et al.*, 2012), yet contrast those of Hansen *et al.* (Hansen *et al.*, 2013) who identified higher follistatin in T2DM patients. The reasons for these discrepancies are not clear at this time and further work is therefore needed to more fully understand the metabolism of follistatin in health and disease.

We report no differences between groups in concentrations of fetuin-A or SeP either in the fasted state or throughout trial days. This may suggest that the development of metabolic complications, and not adiposity *per se*, may be required to disrupt fetuin-A and SeP metabolism. Previous research has found no independent effect of obesity on fetuin-A concentrations (Obuchi *et al.*, 2014), whilst studies reporting differences in SeP have recruited individuals with NAFLD or dysregulated glucose metabolism (Yang, Hwang, *et al.*, 2011; H. Y. Choi *et al.*, 2013).

In the current study we demonstrate that circulating concentrations of FGF21 are increased immediately after an acute 60-min bout of moderate-intensity aerobic exercise, peaking 30 min after the cessation of exercise, and remaining elevated for up to 3 h. A similar, albeit delayed, increase in circulating follistatin also occurred. These findings support previous studies

showing that FGF21 and follistatin are increased with acute aerobic exercise (Slusher *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016). FGF21 and follistatin production are positively regulated by the glucagon to insulin ratio (Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016), whilst FGF21 may also be increased via activation of PPAR-α by circulating NEFA (K. H. Kim *et al.*, 2013; Hansen, Pedersen, *et al.*, 2016). The systemic glucagon to insulin ratio and circulating NEFA were both elevated in response to exercise in the present study. FGF21 improves glucose metabolism in skeletal muscle, adipose tissue and the liver, whilst follistatin may promote pancreatic beta cell survival, reduce circulating glucagon and preserve skeletal muscle mass (Camporez *et al.*, 2013; Hansen, Rutti, *et al.*, 2016). Transient increases in the circulating levels of these hepatokines may represent potential mechanisms which contribute to the short-term improvements in glycaemic control after acute exercise (Sylow *et al.*, 2017) and, if occurring regularly with repeated bouts (i.e. training), the longer-term metabolic benefits associated with regular exercise. Notably, in Wistar rats undergoing a period of HFOF, FGF21 knockout abolished the beneficial effects of exercise training on hepatic steatosis and glucose tolerance (Loyd *et al.,* 2016).

In the current study the responses of circulating FGF21 and follistatin to exercise were similar in both normal weight and overweight/obese participants. It has been previously shown that the FGF21 and follistatin responses to acute exercise are blunted in individuals with T2DM, and this may be the result of differences in the exercise-induced changes in circulating NEFA and the glucagon to insulin ratio (Hansen, Pedersen, *et al.*, 2016). Furthermore, the response of FGF21 to 30 min of exercise at 75% $\dot{V}O_2$ peak has been shown to be reduced in obese individuals compared with healthy, normal weight controls (Slusher *et al.*, 2015). Notably, although the participants in the study by Slusher and colleagues (Slusher *et al.*, 2015) were reportedly healthy, the obese group had a mean HOMA-IR of 4.36, which approaches the 5.13 threshold previously used to distinguish insulin resistant individuals (Wildman *et al.*, 2008). The participants in the current study were free from chronic disease and fasted plasma glucose, insulin and HOMA-IR suggested they were not insulin resistant. It could, therefore, be speculated that exercise-induced increases in circulating FGF21 and follistatin are maintained in overweight/obese individuals with preserved glycaemic control but not once a degree of insulin resistance has developed. Notably, the exercise-induced changes in NEFA and the glucagon to insulin ratio were no different between groups in the current study. This speculative hypothesis, however, should be tested further.

The current study is the first to investigate the acute effects of aerobic exercise on fetuin-A, LECT2 and SeP, but these hepatokines were unaffected by exercise. Fetuin-A, LECT2 and SeP are all negatively regulated by hepatic AMPK (Jung, Choi, *et al.*, 2013; Lan *et al.*, 2014; Trepanowski, Mey and Varady, 2014) which is activated by exercise in an intensity-dependent manner (Camacho *et al.*, 2006). It may be that higher intensity exercise is required to acutely modulate fetuin-A, LECT2 or SeP (Trepanowski, Mey and Varady, 2014), or that repeated bouts of exercise are required to elicit benefits; as shown previously for fetuin-A (Malin, Mulya, *et al.*, 2013; Malin *et al.*, 2014).

This study is not without limitation. Most prominently, this trial was conducted using a relatively small sample of normal weight and overweight/obese men. Given the lack of prior evidence we were unable to determine *a priori* whether our sample size was sufficient for all of our outcomes. The novel data presented in this manuscript may, however, be utilised to inform power calculations for future studies, particularly those investigating the effects of acute exercise on LECT2, SeP and fetuin-A. Our results also cannot be generalised to individuals with chronic metabolic disease or women. Notably, we did not directly measure intrahepatic fat or insulin sensitivity in the current study. These are important considerations because the development of metabolic disease may influence hepatokine metabolism. Furthermore, the heightened propensity for fatty liver development in men, and the potential metabolic influence of sex hormones, underscores the necessity for additional research to be undertaken in women. It should also be noted that all analyses in this study were conducted using plasma isolated from systemic venous blood. Collection of systemic blood has been previously shown to be suitable to assess changes in FGF21 and follistatin after acute exercise (Hansen *et al.*, 2011; Hansen, Pedersen, *et al.*, 2016). However, the potential for changes in other circulating hepatokines (fetuin-A, LECT2 and SeP) to have been missed due to the location or timing of blood sampling cannot be dismissed. Isolation of the splanchnic circulation and the measurement of relevant protein expression in tissues of interest (e.g. hepatic, skeletal muscle and/or adipose tissues) would have been valuable and are necessary to explore this area further in future studies.

In conclusion, this study has identified higher circulating concentrations of FGF21 and LECT2, and lower follistatin, in overweight/obese men when compared to normal weight individuals. Moreover, circulating FGF21 and follistatin are acutely increased after moderate-intensity aerobic exercise, and this beneficial shift in hepatokine profile is similar in both groups. Whilst each of these circulating proteins have been shown to exert beneficial effects of relevance to

metabolic health, the clinical impact of the changes seen with acute exercise in this study is not currently known. These data provide new information regarding the effect of adiposity on the metabolism of several novel hepatokines and supports evidence for a potential role of FGF21 and follistatin in the metabolic benefits associated with exercise (both to a single bout and with regular training). However, additional work is needed to better understand the interaction between these novel proteins, obesity and chronic disease; as well as to better define their interaction with exercise and other metabolic perturbations. Studies exploring the clinical benefit of recurring changes in circulating hepatokines with regular exercise are also required.

CHAPTER 5

THE EFFECTS OF ACUTE MODERATE-INTENSITY CONTINUOUS EXERCISE OR LOW-VOLUME HIGH-INTENSITY INTERVAL TRAINING ON CIRCULATING HEPATOKINE PROFILES IN INDIVIDUALS WITH IMPAIRED GLUCOSE REGULATION

5.1 Abstract

Background: Hepatokines represent a novel mechanism which may mediate the relationship between elevated IHTG and insulin resistance in peripheral tissues. This study presents preliminary findings exploring the effects of acute low-volume high-intensity interval training (LV-HIIT) or continuous moderate-intensity aerobic exercise (CME) on circulating concentrations of three hepatokines in overweight or obese individuals with impaired glycaemic control.

Methods: Six men and six women (median (IQR) or mean ± SD; Age: 69 (67 – 70) years; BMI: 28.8 (28.4 – 31.8) $kg \cdot m^{-2}$; HbA1c: 5.9 ± 0.2 %) performed three experimental trials (control, CME or LV-HIIT), each lasting 6 h, in a randomised, counterbalanced order. Participants performed LV-HIIT (25 min) or CME (35 min) during the respective exercise trials, both of which concluded at 2 h, and rested thereafter. Participants rested throughout control trials. Venous blood samples were collected at 0, 1, 2, 3, 4 and 6 h for the measurement of FGF21, follistatin, fetuin-A, insulin and NEFA.

Results: Circulating follistatin concentrations were greater in the CME trial at 4 h and 6 h, compared to both LV-HIIT and control trials ($P \leq 0.03$). However, there were no differences in circulating follistatin between LV-HIIT and control (all *post hoc* comparisons: $P \geq 0.15$), whilst circulating FGF21 and fetuin-A concentrations were similar across the three trials days (time by trial interaction: $P \geq 0.14$).

Conclusions: These results suggest that, in individuals with impaired glycaemic control, a single bout of CME, but not LV-HIIT, increases circulating concentrations of follistatin, which may contribute to the improved glucose regulation associated with acute and chronic exercise. Conversely, FGF21 and fetuin-A are seemingly unaffected by either CME or LV-HIIT in dysglycaemic individuals. However, additional work is required to explore these preliminary findings further.

5.2 Introduction

Elevated IHTG is the hallmark feature of NAFLD (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018) and is heavily implicated in the pathogenesis of T2DM (Taylor, 2008; Balkau *et al.*, 2010; Armstrong *et al.*, 2014; Mantovani *et al.*, 2018). IHTG correlates with insulin resistance not only in the liver but also in skeletal muscle and adipose tissues (Korenblat *et al.*, 2008; Bril, Barb, *et al.*, 2017). The mechanisms underlying these relationships between IHTG and peripheral tissues are not fully understood but liver-secreted 'hepatokines' may play a part in mediating inter-organ crosstalk (Stefan and Häring, 2013; Takamura, Misu and Kaneko, 2016; Meex and Watt, 2017).

The accumulation of IHTG modulates the secretion of many hepatokines, including FGF21, follistatin and fetuin-A (Meex *et al.*, 2015), each of which have been implicated in the regulation of glucose homeostasis by exerting endocrine effects in skeletal muscle, adipose tissue or the pancreas (Camporez *et al.*, 2013; Malin, Mulya, *et al.*, 2013; Stefan and Häring, 2013; Hansen, Rutti, *et al.*, 2016). Circulating concentrations of FGF21, follistatin and fetuin-A are also higher in patients with T2DM compared to non-diabetic individuals, and correlate with markers of glycaemic control (Chavez *et al.*, 2009; Ishibashi *et al.*, 2010; Chen *et al.*, 2011; Hansen *et al.*, 2013; Stefan *et al.*, 2014). Furthermore, circulating hepatokine concentrations are altered by acute exercise (Hansen *et al.*, 2011; K. H. Kim *et al.*, 2013; Slusher *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016; Sargeant, Aithal, *et al.*, 2018), leading to suggestions that they may be implicated in the metabolic benefits of regular exercise (Slusher *et al.*, 2015).

The study presented in Chapter 4 of this thesis demonstrated that circulating concentrations of FGF21 and follistatin are transiently elevated after a single bout of CME in lean and overweight/obese men (Sargeant, Aithal, *et al.*, 2018), supporting previous research in individuals free from chronic metabolic disease (Hansen *et al.*, 2011; K. H. Kim *et al.*, 2013; Slusher *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016). However, it has been suggested that these responses may be blunted or even abolished in individuals with impaired glycaemic control or T2DM (Slusher *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016). The study in Chapter 4 was also the first to investigate whether circulating concentrations of fetuin-A are modulated by acute exercise. However, despite evidence that circulating fetuin-A may be reduced by exercise training, (which was associated with improved insulin sensitivity) (Malin, Mulya, *et al.*, 2013; Malin *et al.*, 2014), a single bout of CME had no effect (Sargeant,

Aithal, *et al.*, 2018). It is possible that the lack of response in fetuin-A may have been due to the fact that the population recruited were free from chronic disease and had normal glycaemic control. Accordingly, there were no differences in fasted fetuin-A concentrations between lean and overweight/obese groups (Sargeant, Aithal, *et al.*, 2018). No study has explored the effects of acute exercise on circulating fetuin-A in individuals with impaired glucose regulation.

In healthy individuals, the magnitude of increase in circulating FGF21 immediately after a single bout of exercise is dependent upon the intensity of the exercise performed (K. H. Kim *et al.*, 2013). Similarly, fetuin-A expression is negatively regulated by hepatic AMPK activity (Jung, Youn, *et al.*, 2013; Trepanowski, Mey and Varady, 2014), which itself may be activated in an intensity-dependent manner (Camacho *et al.*, 2006). It has also been suggested that the circulating follistatin response to acute exercise may be regulated by exercise intensity (Hansen *et al.*, 2011) but, at present, data from adequately controlled experimental studies are not available. Sustained, continuous high-intensity exercise may not be feasible for individuals with or at risk of chronic metabolic disease. However, LV-HIIT has been proposed as a practical exercise protocol allowing such populations to complete exercise of near-to-maximal intensity (Little *et al.*, 2010, 2011). Whilst evidence remains limited, LV-HIIT has been suggested to improve markers of glycaemic control in overweight individuals with or without T2DM (Hood *et al.*, 2011; Little *et al.*, 2011, 2014; Gillen *et al.*, 2012). However, the effects of LV-HIIT on circulating hepatokines have not been explored.

This chapter presents preliminary analyses from an ongoing clinical trial (ISRCTN12337078) that explores the effects of a single bout of LV-HIIT or CME, on circulating hepatokines (FGF21, follistatin and fetuin-A) in individuals with impaired glycaemic control. It was hypothesised that, LV-HIIT would transiently increase plasma concentrations of FGF21 and follistatin and reduce circulating fetuin-A. Responses to CME would be of a lower magnitude or absent.

5.3 Materials and Methods

5.3.1 Ethical approval and participant recruitment

After receiving approval from the East Midlands NHS Research Ethics Committee (15-EM-0259), 12 white European individuals (six men and six women) were recruited, giving written informed consent to participate (*see Section 3.1*). These participants represent half of the total sample that will be recruited to this study as recruitment of a south Asian group remains in progress. For consistency within this thesis, south Asian individuals recruited to date were not included in these preliminary analyses; all participants in Chapters 4 and 6 were of white European ethnicity.

Participants were aged 50 to 74 years with a BMI > 27.5 $kg \cdot m^{-2}$. Participants were weight-stable (defined as < 5 kg change in body weight in the previous six months) and free from overt chronic metabolic disease but had impaired glycaemic control as indicated by HbA1c between 5.7 and 6.5 %. Participants that performed regular purposeful activity (≥ three or more sessions of vigorous-intensity exercise per week; ≥ 20 min per session) were excluded, as were those taking glucose-lowering medications or steroids.

5.3.2 Preliminary visit

Participants visited the Leicester Diabetes Centre having abstained from alcohol and strenuous exercise for 48 and 72 h, respectively. Height, weight, WC and BF% were measured as outlined in General Methods (*see Section 3.3*). Medical history was reviewed by a specialist cardiac nurse and a resting ECG was performed (Cardiofax GEM ECG, Nihon Nohden Corp., Tokyo, Japan). In the absence of established resting cardiac arrhythmias or other contraindications to participation in the study, participants completed a maximal exercise test to determine $\dot{V}O_2$ peak, as outlined in General Methods (*see Sections 3.6 to 3.8*). ECG was monitored throughout and the test was aborted upon occurrence of any unexpected adverse events.

After approximately 15 min (or until participants had sufficiently recovered), participants were familiarised with the LV-HIIT protocol used during experimental trials. LV-HIIT was performed as outlined in Table 5.1, except that only three intervals were completed. The preliminary visit concluded with a venous blood sample for the measurement of total cholesterol, HDL and HbA1c.

5.3.3 Experimental trials

Participants completed three experimental trials (control, CME and LV-HIIT) in a randomised, counterbalanced order. Each trial was separated by approximately one week and, prior to each trial, participants refrained from strenuous activity and alcohol for 72 h and 48 h, respectively. Participants also recorded dietary intake, using weighed food records, for 48 h before their first trial day and were asked to replicate this prior to subsequent trials. They were instructed to consume all food and energy-containing beverages before 22:00 on the evening before experimental trials, after which only water was permitted.

Participants arrived at the laboratory for experimental trials at approximately 08:00. Motorised transport was arranged and ambulatory activity was minimised throughout trial days. After ensuring compliance with standardisation instructions, experimental procedures were reiterated and willingness to continue was confirmed. An intravenous cannula (20/22G, Braun, Pennine Healthcare, Derby, UK) was inserted into an antecubital vein and, after a period of habituation (30 – 45 min), trials were initiated with the collection of a venous blood sample (0 h). Further blood samples were drawn at 1, 2, 3, 4 and 6 h (Figure 5.1). Circulating FGF21, follistatin, fetuin-A, insulin and NEFA were measured at each timepoint. Standardised meals (8 kcal·kg^{-1}; 51 % CHO, 35 % fat, 14 % protein) were provided at 0 and 3 h, and were consumed within 15 min.

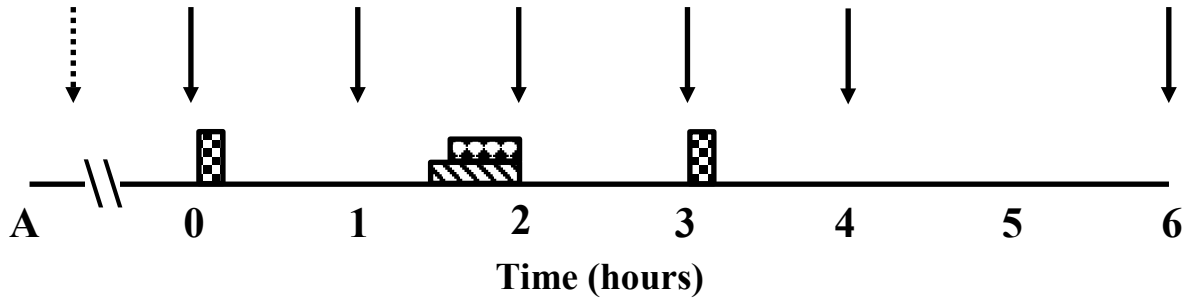


Figure 5.1 Schematic representation of experimental trial days

25 min LV-HIIT performed (LV-HIIT trials only)

35 min of continuous moderate-intensity aerobic exercise performed (CME trials only)

Standardised meal consumed

A Participant arrival (approximately 60 min prior to first blood sample)

Venous cannula inserted (approximately 30 - 45 min prior to first blood sample)

Venous blood sample collected

During exercise trials, participants performed a single bout of either CME or LV-HIIT. Each were performed on a motorised treadmill (Excite Med Technogym, Italy) and finished at 2 h. Therefore, to account for differences in exercise duration (35 vs. 25 min), the CME protocol commenced earlier than the LV-HIIT (Figure 5.1). Details of each exercise protocol, which are suggested to be closely matched for external work (Little *et al.*, 2014), can be found in Table 5.1. During exercise, HR and RPE (Borg, 1970) were recorded at regular intervals.

Table 5.1 Details of exercise protocols employed in experimental trials

	Continuous moderate-intensity exercise	**Low-volume HIIT**
Total duration	35 min	25 min
Warm-up	3.5 km·h^{-1} at 0% gradient (3 min)	
Exercise protocol	Duration: 30 min Speed: Identical to that used in maximal exercise testing Gradient: That predicted to elicit 65 % of $\dot{V}O_2$ peak.	Duration: 20 min (10 x 60-s intervals, 10 x 60-s active recovery). Speed: *Intervals:* Identical to that used in maximal exercise testing. *Recovery:* 3.5 km·h^{-1} Gradient: *Intervals:* 90 % of peak gradient achieved in maximal exercise testing. *Recovery:* 0 %.
Cool-down	3.5 km·h^{-1} at 0% gradient (2 min)	

5.3.4 Biochemical analyses

All biochemical analyses were conducted as outlined in General Methods (*see Sections 3.11 and 3.12*). Briefly, plasma was isolated at each timepoint of experimental trials and stored at -80 ºC (*see Section 3.11*) for subsequent batch analysis of FGF21, fetuin-A, follistatin and insulin using commercially-available ELISA (*see Section 3.12*) (all CV ≤ 5.0%). Glucose, TG, total cholesterol, HDL and HbA1c were requested from the pathology laboratories of University Hospitals of Leicester NHS Trust, whilst NEFA analyses were conducted within

specialist laboratories at Nottingham University Hospitals NHS Trust. One participant was excluded from NEFA analysis as data were unavailable for an entire trial. Haemoglobin and haematocrit were also requested but missing data meant that plasma volume could only be calculated for 64% of all timepoints. Furthermore, complete data for all three experimental trials were only available for two participants. Therefore, no adjustments for plasma volume were made.

5.3.5 Statistical analyses

Data were analysed using an approach that was consistent with that of Chapter 4. Briefly, two-way, repeated measures ANOVA (within-participant factors: trial and sample time) were used to assess hepatokine responses to exercise. Significant trial by time interactions were followed up *post-hoc* with paired samples *t*-tests between the three different trials at each sample time. Given the small sample size of this study, *P*-values were not corrected for multiple comparisons. Total AUC was also calculated for each hepatokine throughout each trial day (*see Section 3.15*) and differences between trials were investigated using one-way repeated measures ANOVA or Friedman's test when the data were normally- or not normally-distributed, respectively. Significant main effects were followed up with paired samples *t*-tests or Wilcoxon matched pairs tests, as appropriate.

5.4 Results

5.4.1 Participant and exercise characteristics

Details of participant characteristics can be found in Table 5.2. As per study eligibility criteria, participants were older adults with overweight or obesity and with HbA1c indicative of impaired glycaemic control. Four of the twelve individuals also met diagnostic criteria for impaired fasting glucose (> 5.7 mmol·L^{-1}) (American Diabetes Association, 2018). There were no differences in HR or RPE between the CME (mean ± SD; HR: 114 ± 17 beats·min^{-1}, RPE: 12 ± 2) or LV-HIIT (HR: 119 ± 12 beats·min^{-1}, RPE: 13 ± 2) exercise (both $P \geq 0.27$).

Table 5.2 Participant characteristics

Anthropometry	
Age (years)	69 (67 – 71)
Body weight (kg)	82.1 ± 9.0
BMI (kg·m^{-2})	28.8 (28.4 – 32.5)
Waist circumference (cm)	101.7 ± 6.4
Body fat (%)	35.7 ± 6.0
Glycaemic Control and Insulin Sensitivity	
HbA1c (%)	5.9 ± 0.2
Fasted plasma glucose (mmol·L^{-1})	5.4 ± 0.5
Fasted plasma insulin (pmol·L^{-1})	72.1 ± 28.3
HOMA-IR	2.9 ± 1.2
Fasted plasma NEFA (mmol·L^{-1})	0.52 ± 0.16
Adipo-IR	35.2 ± 12.5
Other Metabolic Risk Factors	
Systolic blood pressure (mmHg)	135 ± 10
Diastolic blood pressure (mmHg)	81 ± 6
Total Cholesterol (mmol·L^{-1})*	5.2 ± 0.9
HDL (mmol·L^{-1})*	1.6 ± 0.4
LDL (mmol·L^{-1})*	2.6 (2.4 – 2.9)
Fasted plasma TG (mmol·L^{-1})	1.36 (1.19 – 2.31)
Cardiorespiratory Fitness	
Absolute $\dot{V}O_2$ peak (L·min^{-1})	2.19 ± 0.50
Relative $\dot{V}O_2$ peak (mL·kg^{-1}·min^{-1})	26.5 ± 4.6
Fasted Hepatokines	
FGF21 (pg·mL^{-1})	143 (133 – 386)
Fetuin-A (µg·mL^{-1})	682 ± 141
Follistatin (pg·mL^{-1})	2077 ± 480

Data presented as arithmetic mean ± SD or median (IQR). * n = 11.

5.4.2 Circulating hepatokines responses to exercise

FGF21 concentrations were similar between trials (P = 0.79) and there was no interaction between trial and time (P = 0.23; Figure 5.2a). Similarly, there were no differences in the total AUC between trials (Figure 5.2b).

The mean circulating follistatin concentrations were also similar between trials (P = 0.10), but there was a significant interaction between trial and time (P = 0.004; Figure 5.2c). *Post-hoc* tests revealed that, whilst there were no differences between control and LV-HIIT trials (all $P \geq 0.15$), circulating follistatin concentrations were significantly higher at 4 h ($P \leq 0.03$) and 6 h ($P \leq 0.002$) in the CME trial. There was a significant main effect of trial when data were analysed as total AUC (P = 0.05; Figure 5.2d) and *post-hoc* tests revealed a significantly higher total AUC in the CME trial compared to the LV-HIIT trial (P = 0.03). The difference in total AUC between the CME and control trials approached statistical significance (P = 0.08).

Circulating concentrations of fetuin-A were similar between trials (P = 0.67) and there was no interaction between trial and time (P = 0.14; Figure 5.2e). Total AUC was similar between trials (P = 0.50; Figure 5.2f).

5.4.3 Circulating responses of NEFA and insulin to exercise

The mean circulating concentrations of NEFA were similar between trials (P = 0.13) but there was a significant interaction between trial and time (P = 0.02; Figure 5.3a). *Post-hoc* tests revealed that circulating concentrations were higher in the CME trial compared to the control trial at 2 h (P = 0.02) and 3 h (P = 0.02), and higher than the LV-HIIT trial at 1h ($P < 0.01$) and 2 h ($P < 0.001$). The total AUC was similar between trials (P = 0.23; Figure 5.3b).

The interaction between trial and time for circulating concentrations of insulin approached statistical significance (P = 0.08; Figure 5.3c) and there was a main effect of trial when data were analysed either using raw concentrations (P = 0.045) or as total AUC (P = 0.02). *Post-hoc* tests using AUC data suggested that the total AUC during the LV-HIIT trial was lower than that of both the control and CME trials ($P < 0.01$ and P = 0.03, respectively; Figure 5.3d).

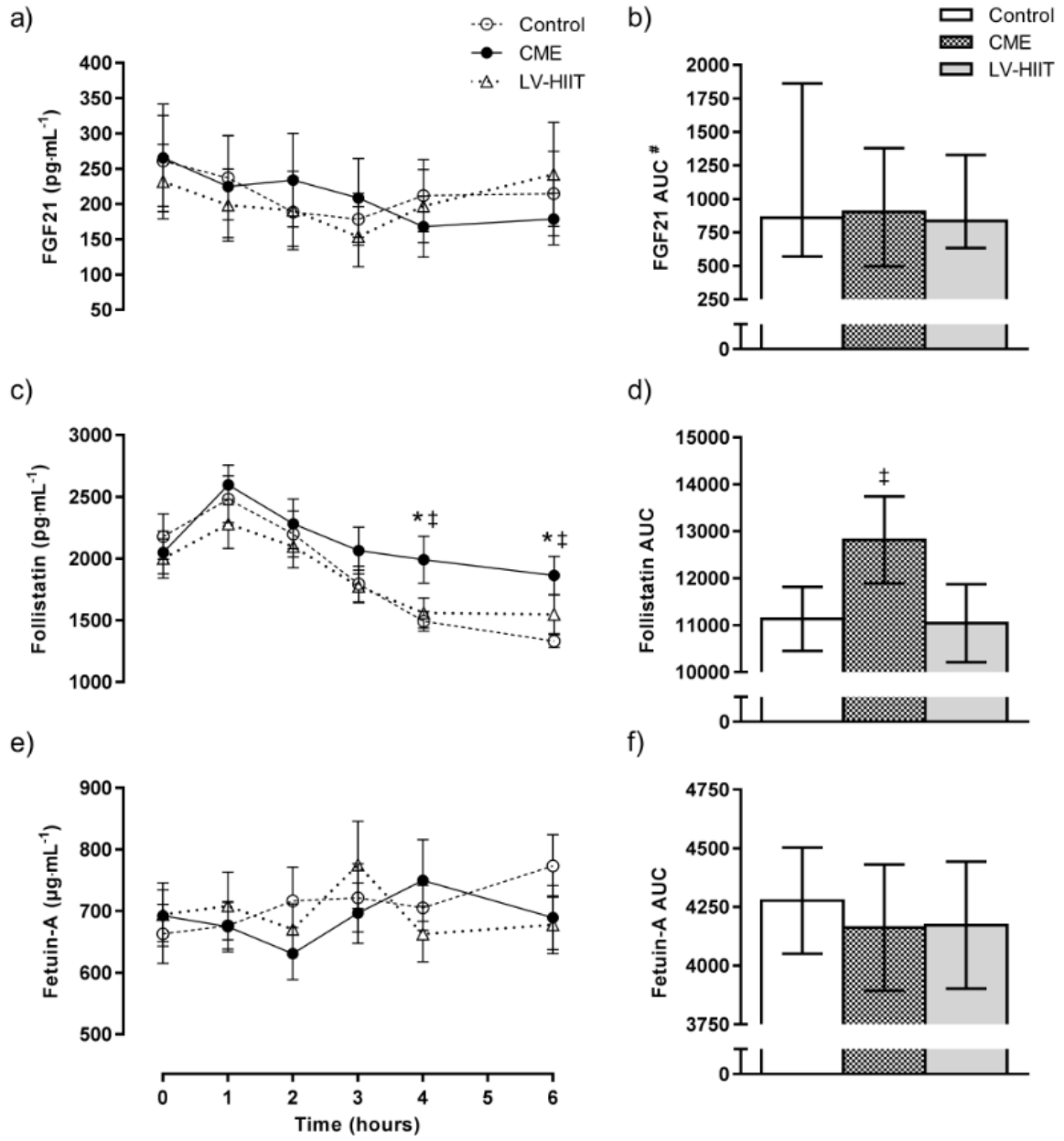


Figure 5.2 Circulating hepatokine response during control, CME and LV-HIIT trials. Circulating plasma concentrations of FGF21 (a-b), follistatin (c-d) and fetuin-A (e-f) during control, CME and LV-HIIT trials. Meals were provided at 0 and 3 h. During CME and LV-HIIT trials, participants performed 35 min of CME or 25 min of LV-HIIT, respectively. Exercise was co-ordinated such that each exercise bout finished at 2 h. Data presented as mean ± SEM unless otherwise stated. # indicates data were not normally distributed and thus presented as median with IQR. AUC represents the total area under the concentration-time curve for the given experimental day. * indicates significant difference between CME and control trials (all $P \leq 0.03$). ‡ indicates significant difference between CME and LV-HIIT trials (all $P \leq 0.01$).

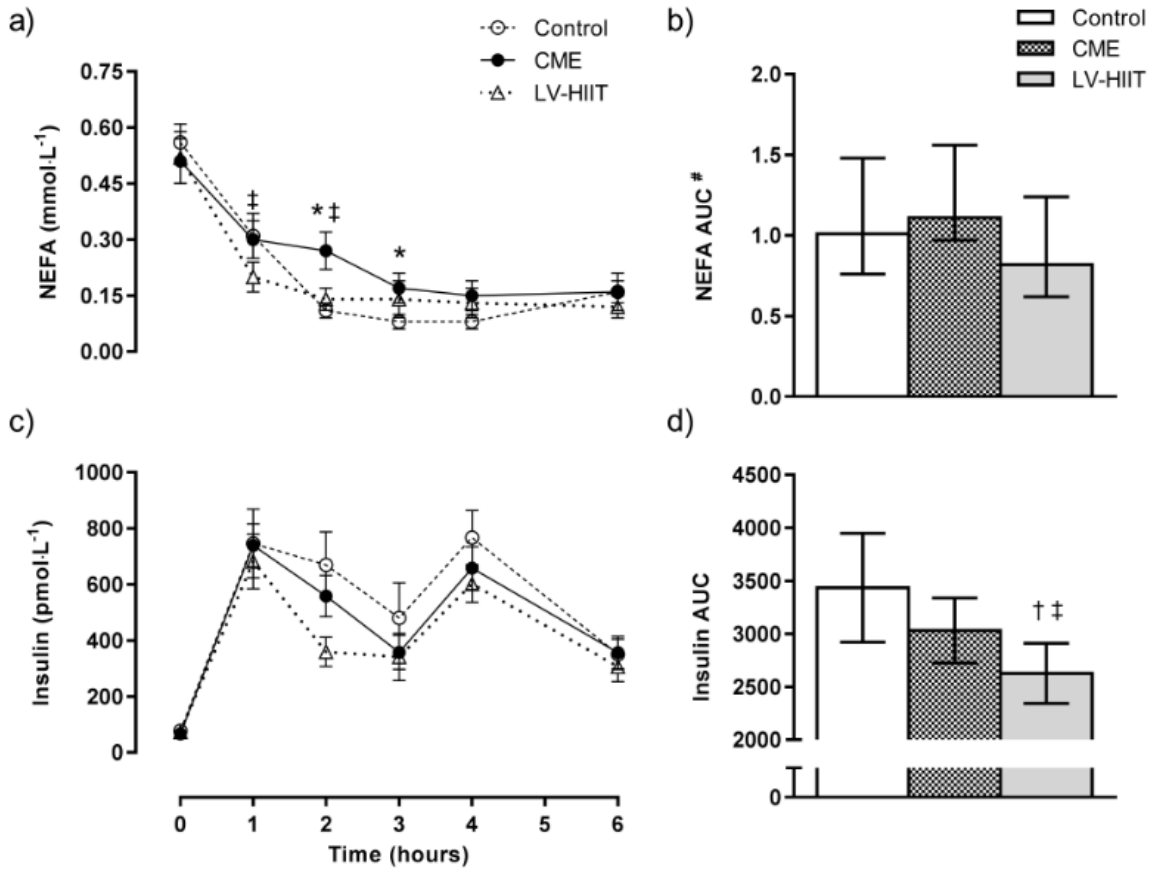


Figure 5.3 Circulating NEFA (a-b) and insulin (c-d) responses during control, CME and LV-HIIT trials. Data for NEFA analysis are n = 11. Meals were provided at 0 and 3 h. During CME and LV-HIIT trials, participants performed 35 min of CME or 25 min of LV-HIIT, respectively. Exercise was co-ordinated such that each exercise bout finished at 2 h. Data presented as mean ± SEM unless otherwise stated. # indicates data were not normally distributed and thus presented as median with IQR. AUC represents the total area under the concentration-time curve for the given experimental day. * indicates significant difference between CME and control trials (all $P \leq 0.02$). ‡ indicates significant difference between CME and LV-HIIT trials (all $P \leq 0.02$). † indicates significant difference between LV-HIIT and control trials ($P = 0.02$).

5.5 Discussion

This chapter presents preliminary findings exploring the acute effects of CME or LV-HIIT on circulating concentrations of FGF21, follistatin and fetuin-A in individuals with overweight or obesity and impaired glycaemic control. These data suggest that circulating follistatin is increased 2 h after the cessation of CME and remains elevated for a minimum of 4 h post-exercise. Conversely, a single bout of LV-HIIT appears to have no effect on circulating follistatin, whilst FGF21 and fetuin-A are unaffected by either exercise bout in this population.

These results are consistent with previous research demonstrating a transient increase in circulating follistatin after a single bout of CME (Hansen *et al.*, 2011; Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016; Sargeant, Aithal, *et al.*, 2018). Notably, one of these studies also reported that this response is blunted in individuals with T2DM (Hansen, Pedersen, *et al.*, 2016). Whilst the reasons for this blunted response could not be determined conclusively, the authors speculated that it may be a result of hyperinsulinaemia in individuals with T2DM, leading to a smaller change in the circulating glucagon to insulin ratio during exercise (Hansen, Pedersen, *et al.*, 2016). This ratio has previously been reported as an important mediator of the post-exercise response of circulating follistatin (Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016). It is notable that in the dysglycaemic population recruited in this study, the mean fasted plasma insulin concentrations was approximately 2 to 2.5-fold greater than both the lean and overweight/obese normoglycaemic groups recruited in Chapter 4 (Sargeant, Aithal, *et al.*, 2018). However, comparisons between different populations are not possible at this time as the current study does not directly compare the magnitude of exercise-induced changes in circulating follistatin between individuals with different degrees of glycaemic control completing the same exercise protocol.

Nonetheless, the data presented in this chapter do confirm the potential for acute exercise to modulate circulating follistatin concentrations in individuals with impaired glucose regulation. Follistatin promotes the synthesis of skeletal muscle, by inhibiting myostatin and activin (Gilson *et al.*, 2009; Yaden *et al.*, 2014), and increases pancreatic β-cell mass by promoting proliferation and reducing apoptosis (Zhao *et al.*, 2015; Hansen, Rutti, *et al.*, 2016). It may also reduce glucagon secretion from islet cells (Hansen, Rutti, *et al.*, 2016). It is plausible, therefore, that regular transient elevations in follistatin after repeated bouts of exercise may play a part in the improvements in glycaemic control seen with exercise training. However, this hypothesis requires further investigation.

Previous reports have speculated that the response of circulating follistatin to acute exercise may be dependent on the intensity of exercise performed (Hansen *et al.*, 2011). In the current analysis, however, LV-HIIT had no effect on circulating follistatin concentrations. Whilst contrary to the hypothesis of Hansen and colleagues, these results are consistent with a separate study recently conducted in our research group (Willis *et al.*, 2018). This study demonstrated that, in healthy normal-weight individuals, the exercise-induced increase in circulating follistatin was no different after energy-matched bouts of aerobic exercise performed at either 55% or 75% of $\dot{V}O_2$ peak (Willis *et al.*, 2018). It is possible, therefore, that characteristics of acute exercise other than intensity may be more important in mediating the post-exercise increase in circulating follistatin. One such exercise characteristic may be exercise duration. In the study by Hansen and colleagues, the increase in circulating follistatin was greater after 3 h of moderate-intensity cycling than after 2 h of knee extensor exercise (Hansen *et al.*, 2011). Similarly, in the current study the single bout of CME was approximately one-third longer than the LV-HIIT.

An alternative explanation for the lack of change in circulating follistatin after LV-HIIT is that the LV-HIIT utilised in this study did not induce changes in the glucagon to insulin ratio sufficient to modulate hepatic follistatin secretion. As mentioned previously, the circulating follistatin response after acute exercise is regulated by changes in the glucagon to insulin ratio and when exercise is performed under the conditions of a pancreatic clamp (during which glucagon and insulin concentrations are fixed) the post-exercise increase in plasma follistatin is blunted (Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016). Glucagon was not measured in the current analyses and, therefore, it cannot be determined at present whether the glucagon to insulin ratio differed between the CME and LV-HIIT trials. It is noteworthy, however, that the mean circulating insulin concentration was lower during the LV-HIIT trial compared to the CME and control trials. This would, if anything, contribute to a greater glucagon to insulin ratio. Finally, the fact that the follistatin response to acute exercise is blunted but not abolished during pancreatic clamp suggests that additional mechanisms beyond the glucagon to insulin ratio may be responsible. Whilst, these alternative mechanisms are currently unclear it is possible that they may be differentially modulated by CME and LV-HIIT.

In the current study, circulating concentrations of FGF21 were unaffected by CME in individuals with impaired glucose regulation but not overt T2DM. Furthermore, despite previous evidence that the post-exercise increase in circulating FGF21 may be intensity-dependent in healthy individuals (K. H. Kim *et al.*, 2013), a single bout of LV-HIIT in the

current study had no effect. These results are in accordance with previous research demonstrating that, compared to healthy normoglycaemic individuals, the increase in circulating FGF21 after exercise is blunted, or even abolished, in individuals with dysregulated glucose metabolism (Slusher *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016). Notably, the individuals recruited by Slusher and colleagues were obese and had a baseline HOMA-IR that was indicative of insulin resistance, but were free from chronic metabolic disease (Wildman *et al.*, 2008; Slusher *et al.*, 2015); HbA1c was not assessed. Collectively, these results indicate that there may be a progressive decline in the FGF21 response to exercise as insulin resistance occurs and this may be fully suppressed before the development of overt T2DM. As such, changes in FGF21 may not be directly related to the acute improvement in insulin sensitivity seen after single bouts of exercise in individuals with dysglycaemia (Sylow *et al.*, 2017). However, as mentioned previously when discussing the results for follistatin above, further studies directly comparing the response of FGF21 to identical bouts of acute exercise in matched groups of individuals with different degrees of insulin resistance are required to rigorously test this suggestion.

The circulating FGF21 response to exercise is also regulated by the glucagon to insulin ratio (Hansen *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016). However, unlike follistatin, this response is completely abolished when exercise is performed under conditions of the pancreatic clamp (Hansen, Pedersen, *et al.*, 2016). It has been previously suggested that the blunted or abolished response of circulating FGF21 after acute exercise in individuals with impaired glycaemic control or overt T2DM may be due to elevated circulating insulin in these individuals leading to a lower change in the glucagon to insulin ratio with exercise (Hansen, Pedersen, *et al.*, 2016). As mentioned previously, however, glucagon was not measured in the current study and, therefore, whilst fasted circulating insulin was greater in the current study population compared to the normoglycaemic individuals recruited in Chapter 4, potential differences in the glucagon to insulin ratio cannot be determined.

The effects of acute exercise on circulating fetuin-A in individuals with impaired glucose regulation have not been explored previously. The preliminary findings presented in this chapter suggest that, in this population, plasma fetuin-A concentrations are unaffected by a single bout of either CME or LV-HIIT. These findings are similar to those previously reported in lean and overweight/obese individuals with normal glucose regulation, which also found no effect of acute CME on circulating fetuin-A (Sargeant, Aithal, *et al.*, 2018; Chapter 4 of this thesis). It was hypothesised that the lack of fetuin-A response to acute exercise in individuals

free from chronic metabolic disease (Sargeant, Aithal, *et al.*, 2018) may have been because fetuin-A concentrations were not elevated at baseline. In Chapter 4, there was no difference in circulating fetuin-A between the lean and overweight/obese normoglycaemic groups and this may have limited the potential for circulating fetuin-A to be reduced by acute exercise. Alternatively, fetuin-A expression is negatively regulated by hepatic AMPK (Jung, Youn, *et al.*, 2013; Trepanowski, Mey and Varady, 2014) which, in rodents, is activated in a stepwise manner with exercise intensity (Camacho *et al.*, 2006). Therefore, it may have been that the intensity of CME utilised in Chapter 4 was insufficient to modulate circulating fetuin-A. However, despite the mean fasted fetuin-A concentration in the current study being 26% to 37% higher than the groups recruited in Chapter 4 and a bout of high-intensity exercise being performed in the form of LV-HIIT, there was no effect of either CME or LV-HIIT on circulating fetuin-A. Therefore, the results of the current study in combination with those of Chapter 4 (Sargeant, Aithal, *et al.*, 2018) suggest that, in humans, fetuin-A may not be sensitive to acute perturbations in metabolism, such as that caused by a single bout CME or LV-HIIT. Instead, it may be that more regular bouts of energy imbalance, such as those that occur with structured training, or prolonged energy deficit and weight loss may be required to elicit sustained reductions in circulating fetuin-A (Blüher *et al.*, 2012; Malin, Mulya, *et al.*, 2013; Malin *et al.*, 2014).

This study is the first to investigate the effects of a single bout of LV-HIIT or CME on circulating hepatokines in overweight/obese individuals with impaired glycaemic control but not overt T2DM. However, some important limitations should be considered. Most notably, this chapter presents preliminary analyses of an ongoing trial, with the current sample size constituting half of the final study population. As such, the current sample size may be insufficient to detect physiologically meaningful changes in some of the outcomes reported and null-findings may be the result of a lack of statistical power. Notably, based on the data contained in this chapter, where the largest standardised difference between trials for total AUC of FGF21 (between CON and CME) and fetuin-A (between CON and LV-HIIT) were -0.18 and -0.20, respectively, and where the within-participant correlation was approximately 0.90 for each, the current sample size (n = 12) only had 27.5% and 20.8% power to detect a significant main effect of trial, with an alpha error rate of 0.05. In turn, retrospective sample size calculations determined that 46 and 67 participants would be required to detect a significant main effect of trial for FGF21 and fetuin-A, respectively. Furthermore, additional research examining the impact of transient exercise-induced changes in FGF21 and fetuin-A

in clinical populations is required to determine what magnitude of difference in these hepatokines could be considered clinically meaningful. Accordingly, it is important to note that the potential explanations provided for the results of this chapter remain speculative and require rigorous investigation. Secondly, participants performed exercise approximately 60-90 min after consuming a breakfast meal. Whilst the content and timing of meals were standardised between conditions, and thus controlled within the context of this study, direct comparisons with previous studies (in which exercise was performed in the fasted state) should be made with caution. Finally, the CME and LV-HIIT protocols utilised in this study are suggested to be approximately matched for total external work (Little *et al.*, 2014) and the mean HR and RPE during exercise was similar between trials. However, energy expenditure was not assessed directly (using indirect calorimetry, for example, as it was in Chapter 4) and therefore the possibility that differences in responses between CME and LV-HIIT may be due to differences in energy expenditure cannot be dismissed. Furthermore, it may be possible that the exercise protocols used in this chapter were insufficient to elicit changes in circulating hepatokines and that greater energy expenditure may be required to modulate circulating concentrations in this population. Further studies that rigorously examine the impact of exercise protocols differing in energy expenditure are required to test this hypothesis, and explore whether a minimum threshold of energy expenditure required to elicit changes in circulating hepatokines exists.

In conclusion, this study demonstrates that circulating follistatin is transiently increased by a single bout of CME in individuals with overweight or obesity and impaired glycaemic control, which may contribute to the improved glucose regulation associated with regular exercise. Conversely, the data presented suggest that the increase in FGF21 previously reported after CME in a healthy (normoglycaemic) population is not apparent in individuals with glycaemic dysregulation. A single bout of LV-HIIT had no effect on follistatin or FGF21, whilst circulating concentrations of fetuin-A were unaffected by either exercise protocol. However, these analyses are preliminary in nature and additional work is required to explore these findings further.

CHAPTER 6

EFFECTS OF SPRINT INTERVAL TRAINING ON ECTOPIC LIPIDS AND TISSUE-SPECIFIC INSULIN SENSITIVITY IN MEN WITH NON-ALCOHOLIC FATTY LIVER DISEASE

The study presented in this chapter has been published and has the following citation:

Sargeant JA, Bawden S, Aithal GP *et al*. (2018). *European Journal of Applied Physiology.* **118**(4): 817-828.

This chapter presents the published manuscript but with some aspects of the methods section condensed or removed to avoid repetition within this thesis. When this occurs, readers are directed to the appropriate sections of the General Methods for more information. Conversely, further details of the participant recruitment process and hepatokines analyses, which were not submitted for publication, have been added to the results section of this chapter.

6.1 Abstract

Purpose: This study examined the feasibility of sprint interval exercise training (SIT) for men with non-alcoholic fatty liver disease (NAFLD) and its effects on intrahepatic triglyceride (IHTG), insulin sensitivity (hepatic and peripheral), visceral (VAT) and subcutaneous adipose tissue (ScAT).

Methods: Nine men with NAFLD (age 41±8 years; BMI 31.7±3.1 $kg \cdot m^{-2}$; IHTG 15.6±8.3%) were assessed at: 1) baseline 2) after a control phase of no intervention (pre-training) and 3) after six weeks of SIT (4-6 maximal 30 s cycling intervals, three times per week). IHTG, VAT and ScAT were measured using magnetic resonance spectroscopy or imaging and insulin sensitivity was assessed via dual-step hyperinsulinaemic-euglycaemic clamp with [6,6-D2] glucose tracer.

Results: Participants adhered to SIT, completing ≥96.7% of prescribed intervals. SIT increased peak oxygen uptake ($\dot{V}O_2$ peak: +13.6% [95% CI: 8.8 to 18.2%]) and elicited a relative reduction in IHTG (-12.4% [-31.6 to 6.7%]) and VAT (-16.9% [-24.4 to -9.4%]; n=8), with no change in body weight or ScAT. Peripheral insulin sensitivity increased throughout the study (n=8; significant main effect of phase) but changes from pre- to post-training were highly variable (range: -18.5 to +58.7%) and not significant (P=0.09), despite a moderate effect size (g*=0.63). Hepatic insulin sensitivity was not influenced by SIT.

Conclusions: SIT is feasible for men with NAFLD in a controlled laboratory setting and is able to reduce IHTG and VAT in the absence of weight loss.

6.2 Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common complication of obesity that is integrated in the pathogenesis of extra-hepatic co-morbidities such as type 2 diabetes (T2DM) and cardiovascular disease (Byrne and Targher, 2015). Insulin resistance is a central pathophysiological feature of NAFLD with associations between intrahepatic triglyceride content (IHTG) and insulin action in skeletal muscle, adipose tissue and the liver (Bril, Barb, *et al.*, 2017). The prominence of these metabolic defects within the development and progression of NAFLD makes them priority targets for intervention.

Lifestyle interventions, incorporating diet and physical activity, remain the cornerstone of treatment for NAFLD (Marchesini *et al.*, 2016) and the importance of structured exercise within such interventions is underscored by both hepatic and extra-hepatic benefits. Continuous moderate-to-vigorous intensity exercise interventions increase cardiorespiratory fitness, reduce adiposity, improve peripheral insulin sensitivity, enhance cardiovascular function and improve circulating markers of metabolic health (Pugh *et al.*, 2014; Hallsworth *et al.*, 2015; Keating *et al.*, 2015; Cuthbertson *et al.*, 2016; Zhang *et al.*, 2016).

Guidelines for the management of NAFLD suggest that individuals undertake 150-200 min of moderate-intensity aerobic or resistance exercise each week, spread over three to five sessions (Marchesini *et al.*, 2016). Observational evidence (Kistler *et al.*, 2011) and experimental data (Cho *et al.*, 2015; Oh *et al.*, 2017) suggest that high-intensity exercise may be more potent in attenuating IHTG accumulation and NAFLD progression than moderate-intensity exercise. This evidence is consistent with exercise intensity-dependent improvements in wider cardiometabolic outcomes, including indices of insulin sensitivity (Tjønna *et al.*, 2008; Weston, Wisløff and Coombes, 2014). Moderate-intensity exercise improves peripheral insulin sensitivity in patients with NAFLD (Cuthbertson *et al.*, 2016) but evidence of its impact on hepatic insulin sensitivity is unclear (Shojaee-Moradie *et al.*, 2007; Cuthbertson *et al.*, 2016). The effects of high-intensity exercise on hepatic and peripheral insulin sensitivity in individuals with NAFLD have not been assessed. A better understanding of these outcomes is important, given the link between IHTG, glycaemic control and metabolic disease (Byrne and Targher, 2015; Marchesini *et al.*, 2016; Bril, Barb, *et al.*, 2017).

High-intensity intermittent exercise training (HIIT), which is characterised by repeated intervals of high-intensity exercise interspersed with periods of rest or low-intensity active recovery, has emerged as a form of exercise capable of providing many health benefits for

individuals with, or at risk of, chronic disease (Gibala *et al.*, 2012). Sprint interval training (SIT) is a version of HIIT consisting of brief bursts (30 s) of maximal-intensity exercise (Little *et al.*, 2011). SIT induces adaptations in skeletal muscle which improve oxidative metabolism (Gibala *et al.*, 2012) and, in some studies, enhances whole-body insulin sensitivity and glycaemic control (Richards *et al.*, 2010; Cocks *et al.*, 2015). These adaptations are likely to be of benefit for individuals with NAFLD, but the influence of SIT on IHTG and tissue-specific (muscle, adipose tissue, liver) insulin sensitivity remains unknown.

This study investigated the feasibility and efficacy of SIT as a therapeutic strategy in overweight or obese men with NAFLD. We sought to determine the effect of six weeks of SIT on IHTG, visceral (VAT) and subcutaneous adipose tissue (ScAT), as well as hepatic and peripheral (skeletal muscle and adipose tissue) insulin sensitivity. We hypothesised that SIT would reduce IHTG, VAT and ScAT, and increase insulin sensitivity.

6.3 Participant and Methods

6.3.1 Ethical approval

This study was approved by the research ethics committees of Loughborough University and the University of Nottingham and was conducted in accordance with the Declaration of Helsinki (World Health Organisation, 2013).

6.3.2 Participants

Nine white European men were recruited from the general population and gave informed, written consent to participate (*see Section 3.1*). Although no power calculation was performed for this study, this sample size was chosen based on studies reporting significant improvements in cardiorespiratory fitness and indices of glycaemic control with HIIT (Little *et al.*, 2011; Cocks *et al.*, 2015). Eligibility criteria included inactive but weight-stable individuals aged 25 to 55 years with a body mass index (BMI) between 27 and 40 $kg \cdot m^{-2}$ and a waist circumference ≥ 94 cm. Participants were considered inactive if they did not complete any form of regular structured exercise. Participants were identified as exhibiting NAFLD in that they had IHTG ≥ 5.56%, determined during screening using magnetic resonance (MR) spectroscopy (^{1}H-MRS), and did not report excessive alcohol consumption (>21 $units \cdot week^{-1}$; Appendix VIII) or other secondary causes of hepatic steatosis (Marchesini *et al.*, 2016). Participants were excluded if they: a) had any form of diagnosed chronic metabolic disease, b) were taking prescribed medications for hypertension, dyslipidaemia or glucose regulation or c) had contraindications to exercise or MR procedures.

6.3.3 Study design

This study utilised a repeated measures longitudinal design in which, following screening, participants completed two consecutive six week phases (control and SIT). This study design was chosen over an RCT design to reduce the number of participants recruited and the number of measurement sessions required. Our within-measures design also meant that we avoided additional heterogeneity that would occur through the recruitment of different individuals into two separate groups. Notably, our control phase acted to monitor the variation in study outcomes over a similar period to that of the exercise intervention, but with participants maintaining their usual lifestyle. All participants completed the control phase followed by the exercise intervention, in order to avoid the confounding effects of detraining during the control phase. All study assessments were performed on consecutive days at baseline, pre-training and

post-training: day 1) IHTG, VAT and ScAT; day 2) hepatic and peripheral insulin sensitivity and systemic metabolic biomarkers; day 3) cardiorespiratory fitness. Post-training assessments began 48 h after the final SIT session to eliminate the confounding influence of the final exercise training session on insulin sensitivity (Sylow *et al.*, 2017).

Dietary intake was standardised for 24 h before metabolic assessments through provision of all food and energy-containing drinks. This diet provided a balanced macronutrient profile and was tailored to each participant's estimated energy requirement (Mifflin *et al.*, 1990) using a multiplication factor of 1.45 to account for the physical activity level of an inactive group (FAO, WHO and UNU, 2001). Participants were instructed and regularly reminded to maintain their usual lifestyle habits throughout both the control and SIT phases of the study. This included instructions to maintain dietary habits. Energy intake was not recorded due to concerns that monitoring may prompt dietary changes and given documented concerns regarding the accuracy of self-reported energy intake data (Dhurandhar *et al.*, 2015).

6.3.4 Imaging and metabolic assessments

All metabolic assessments were performed after an overnight fast. MR measurements were performed on a Philips Achieva 3T system with 32 channel XL-Torso coil. IHTG was measured from a 20x20x20 mm voxel within the right lobe of the liver using ^{1}H-MRS with Stimulated Echo Acquisition Mode (STEAM) localization (repetition time = 2046 ms) (Stephenson *et al.*, 2013; Bawden, Scott and Aithal, 2017). VAT and ScAT were assessed using a two-point modified Dixon technique (Philips) (Nakai *et al.*, 2010) and an in-house algorithm to generate fat boundaries of visceral and subcutaneous regions.

Hepatic and peripheral insulin sensitivity were assessed using a modified version of the hyperinsulinaemic, euglycaemic clamp with two stages of insulin infusion, low- (20 $mU \cdot m^{-2} \cdot min^{-1}$) and high-dose (50 $mU \cdot m^{-2} \cdot min^{-1}$), each lasting 120 min. A continuous infusion of [6,6-D2] glucose tracer was initiated 120 min before the first hyperinsulinaemic stage and continued throughout for the quantification of endogenous glucose production (EGP) (Johnston *et al.*, 2013). Blood glucose was clamped at 4.5 $mmol \cdot L^{-1}$ (coefficient of variation: mean (± SD) = 1.6 ± 0.9 and 2.7 ± 1.1 % at steady-state low- and high-dose insulin infusion, respectively). Further details of ^{1}H-MRS, MRI and dual-step hyperinsulinaemic, euglycaemic clamp procedures can be found in the General Methods section of this thesis (*see Sections 3.9 and 3.10*).

The hepatic insulin sensitivity index (HISI) (Matsuda and DeFronzo, 1999) and the percentage suppression of EGP by low-dose insulin infusion ($\%EGP_{supp}$) were calculated as indices of hepatic insulin sensitivity in the fasted and insulin-stimulated states, respectively, as outlined in General Methods (*see Section 3.14*). Peripheral insulin sensitivity was assessed as whole-body glucose uptake, which was assumed to be equal to the exogenous glucose infusion rate required to maintain euglycaemia at high-dose insulin infusion, during which EGP was negligible.

6.3.5 Assessment of cardiorespiratory fitness and habitual physical activity

$\dot{V}O_2$ peak and PPO were measured using a ramped (+16 Watt·min^{-1}) cycling test on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode BV, The Netherlands) (*see Sections 3.6 to 3.8 of General Methods for further details*). $\dot{V}O_2$ peak and PPO are presented as both absolute units (L·min^{-1} and W) and relative to the participant's body weight (mL·kg^{-1}·min^{-1} and W·kg^{-1}). Participants were familiarised with this test one week before baseline assessments.

To assess the impact of SIT on habitual physical activity levels, participants wore a tri-axial accelerometer (GT3x, Actigraph LLC, USA) for seven consecutive days before each assessment (baseline, pre-training and post-training). Data were analysed using computer software (Kinesoft 3.3.80, USA) (Troiano *et al.*, 2008) and are presented as absolute minutes per day in each activity domain (sedentary time, light, moderate and vigorous physical activity) as well as percentages of accelerometer wear time. Further details of data collection and analysis can be found in Appendix VIII)

6.3.6 Exercise training

Participants completed a SIT program consisting of three exercise sessions per week for six weeks. Sessions consisted of a low-intensity warm-up (5 min cycling at 50W), followed by 30 s intervals of maximal sprint cycling on a stationary ergometer (Ergomedic 894E, Monark Exercise AB, Sweden), which was separated by periods of active recovery (4.5 min of low intensity cycling at 50W). The braking resistance of the ergometer was increased during intervals through the application of a load equivalent to 6.5% of lean body mass, determined using bioelectrical impedance analysis (BC-418, TANITA Europe BV, Amsterdam, The Netherlands). Participants were instructed to cycle 'all-out' during intervals whilst members of the research team provided verbal encouragement (Whyte, Gill and Cathcart, 2010). Four

intervals were completed per session in the first two weeks with an additional interval added to each session every two weeks. Participants therefore completed 90 intervals over the six weeks of supervised training.

6.3.7 Biochemical analyses

Plasma glucose isotope enrichment (atoms percent excess) was quantified as the oxime/trimethylsilyl derivative via gas chromatography mass spectrometry (GC-MS; 7890B, MSD 5977A; Agilent Technologies, UK) using selected ion monitoring of the ions at m/z 319 and 321 (CV = 6.4%). Fasted circulating concentrations of total cholesterol, HDL, LDL, TG and NEFA (All CV ≤ 1.5%), and FGF21, follistatin, fetuin-A and LECT2 (all CV ≤ 7.1%) were analysed from plasma aliquots, collected before the start of hyperinsulinaemic euglycaemic clamps (*as outlined in General Methods, Sections 3.11 & 3.12*) (All CV ≤ 1.5%). Serum insulin was quantified using radioimmunoassay (Millipore, USA) (CV = 7.3%), and HOMA-IR and Adipo-IR were calculated (*see Section 3.14*) (Matthews *et al.*, 1985; Gastaldelli *et al.*, 2007).

6.3.8 Tracer calculations

Rates of EGP in the basal state and at low-dose insulin infusion were calculated (Wolfe and Chinkes, 2005; Vella and Rizza, 2009), accounting for non-steady-state during low-dose insulin infusion and assuming a fractional volume of distribution of 160 $mL \cdot kg^{-1}$. Further details of glucose tracer calculations can be found in Appendix VIII.

6.3.9 Statistical analyses

Normally distributed data are presented as mean with standard deviation (SD) and one-way repeated measures ANOVA was used to assess changes in outcomes across assessment visits (main effect of phase). The homogeneity of variance between data collected at each visit was assessed and a Greenhouse-Geisser or Huynh-Feldt correction was applied, where appropriate. Statistically significant main effects were explored *post-hoc* using paired samples *t*-tests. Non-normally distributed data are presented as the median with interquartile range (IQR) and Friedman tests were used to assess the main effect of phase. Wilcoxon matched pairs tests were used for *post-hoc* pairwise comparisons on non-normally distributed data. Probability (*P*-) values for *post-hoc* tests were adjusted using the Holm-Bonferroni correction (Holm, 1979) to account for multiple comparisons. In text, the changes from pre- to post-training are presented as relative percentage change along with 95% confidence interval [CI] and effect size (adjusted Hedges' *g**). Cohen's descriptors were used to interpret the magnitude of effect (Cohen, 1988).

For clarity, the change in IHTG is presented as both the absolute and relative percentage change. The association between changes in IHTG and whole-body glucose uptake from pre- to post-SIT was assessed using Pearson's bivariate correlation analysis.

6.4 Results

6.4.1 Participant recruitment

One-hundred and thirty-five men expressed an interest to participate and underwent pre-screening via face-to-face, telephone or email contact. Of these, 32 progressed to formal screening, nine of whom were eligible for inclusion in the study. A flowchart of study recruitment, along with reasons for exclusion, can be found in Figure 6.1.

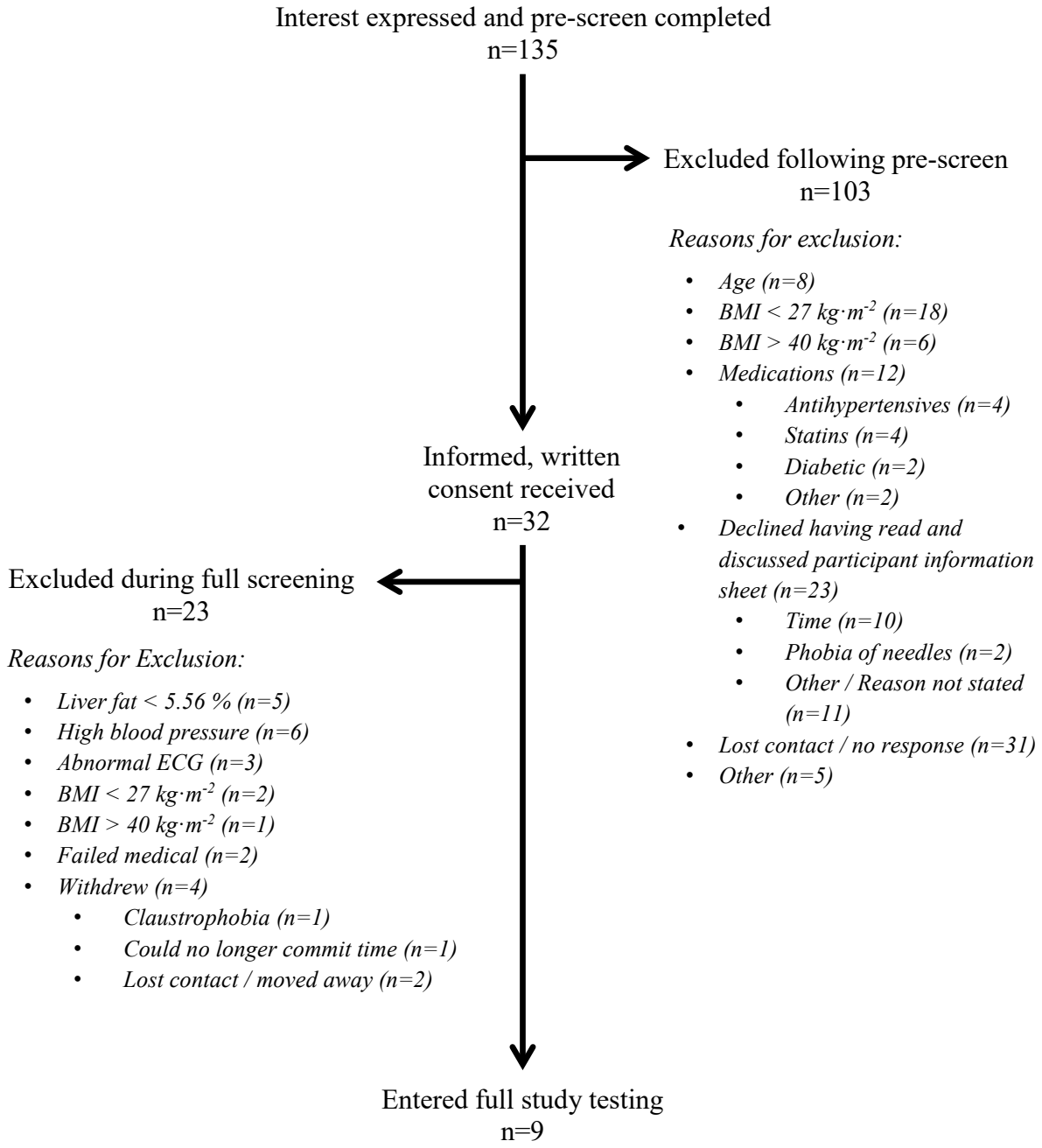


Figure 6.1 Study recruitment process

6.4.2 Participant characteristics and exercise training compliance

All assessments made at baseline, pre- and post-training can be found in Table 6.1. The median self-reported alcohol intake of recruited participants was four units per week (range: 1 to 14). One participant was unable to attend hyperinsulinaemic, euglycaemic clamp assessments. Participant characteristics specific to these eight individuals can be found in Appendix IX. All participants completed exercise training and attended all 18 sessions. Due to fatigue, one participant failed to complete two intervals in their first session and one interval in session two, but completed all prescribed intervals thereafter (total intervals: 87 = 96.7%).

There were no significant differences in any measured outcome between baseline and pre-training assessments, determined as a non-significant main effect of phase or, where appropriate, *post-hoc* comparison. However, from baseline to pre-training assessments, there was a tendency for increased fasted serum insulin and HOMA-IR, and reduced relative $\dot{V}O_2$ peak (uncorrected $P = 0.06$, 0.07 and 0.07, respectively; all other outcomes $P \geq 0.13$).

6.4.3 Effects of SIT on cardiorespiratory fitness and habitual physical activity

Training improved absolute and relative $\dot{V}O_2$ peak by 11.2% [95% CI: 6.4 to 16.0%] ($g^* = 0.83$) and 13.6% [8.8 to 18.2%] ($g^* = 0.78$; Figure 6.2a), respectively ($P \leq 0.001$). This was alongside improvements in absolute (14.7% [10.7 to 18.7%], $g^* = 0.75$) and relative (16.2% [11.1 to 21.2%], $g^* = 0.64$; Figure 6.2b) peak power output ($P \leq 0.001$). As outlined in Table 6.2, there were no differences in sedentary time or light, moderate or vigorous physical activity throughout the duration of the study when analysed either as minutes per day or as a percentage of accelerometer wear time (main effect of phase: $P \geq 0.24$).

Table 6.1 Participant characteristics and study outcomes (continued overleaf)

	Baseline	*Pre-training*	*Post-training*	*P-value (main effect of phase)*	*Effect size (Hedges' g*)*	
					Baseline-Pre-Training	*Pre- to Post-Training*
Anthropometry						
Age (years)	41 ± 8					
BMI (kg·m^{-2})	31.7 ± 3.1					
Waist circumference (cm)	111.3 ± 7.5					
Body weight (kg)	102.5 ± 10.6	102.4 ± 10.1	101.2 ± 10.4	0.17	Trivial	Trivial
VAT (mL) (*n=8*)	1094 ± 238	1220 ± 395	995 ± 281	**0.01**	0.36	-0.62
ScAT (mL) (*n=8*)	2801 ± 847	2800 ± 653	2658 ± 655	0.16	Trivial	-0.21
IHTG (%)	15.6 ± 8.4	14.4 ± 8.7	12.4 ± 8.2	**0.001**	Trivial	-0.23
Cardiorespiratory Fitness						
Absolute $\dot{V}O_2$ peak (L·min^{-1})	3.23 ± 0.41	3.18 ± 0.41	3.53 ± 0.45[c]	**<0.001**	Trivial	0.83
Relative $\dot{V}O_2$ peak (mL·kg^{-1}·min^{-1})	31.8 ± 4.8	31.0 ± 4.7[a]	35.1 ± 5.4[c]	**<0.001**	Trivial	0.78
Absolute peak power output (W)	239 ± 42	237 ± 42	270 ± 43[d]	**<0.001**	Trivial	0.75
Relative peak power output (W·kg^{-1})	2.36 ± 0.49	2.34 ± 0.54	2.71 ± 0.57[d]	**<0.001**	Trivial	0.64
Insulin Sensitivity (n=8)						
Fasted serum insulin (mU·L^{-1})	17.6 ± 4.5	21.8 ± 8.1[a]	18.2 ± 6.0[b]	**0.04**	0.60	-0.48
Fasted blood glucose (mmol·L^{-1})	4.7 ± 0.3	4.7 ± 0.4	4.5 ± 0.5	0.13	Trivial	-0.32
HOMA-IR	3.7 ± 1.0	4.5 ± 1.7[a]	3.7 ± 1.2[b]	**0.03**	0.59	-0.56
Whole-body glucose uptake (mg·kg^{-1}·min^{-1})	5.2 ± 1.1	5.4 ± 1.1	6.4 ± 1.8[e]	**0.02**	Trivial	0.63
Fasted plasma NEFA (mmol·L^{-1})	0.59 ± 0.15	0.55 ± 0.12	0.58 ± 0.19	0.83	-0.41	0.29

Adipo-IR*	52.7 (44.6 – 83.2)	69.3 (46.9 – 101.8)	55.4 (45.8 – 73.4)	>0.99	0.38	-0.24
Basal EGP (μmol·kg^{-1}·min^{-1})	10.8 ± 1.5	10.7 ± 2.2	11.2 ± 2.1	0.70	Trivial	0.25
HISI* (mg·m^{-2}·min^{-1} per mU·L^{-1})	0.65 (0.54 – 0.76)	0.54 (0.50 – 0.62)	0.63 (0.49 – 0.74)	0.42	-0.47	0.34
Low-dose EGP (μmol·kg^{-1}·min^{-1})	4.39 ± 2.17	4.63 ± 1.31	5.34 ± 1.58	0.27	Trivial	0.46
%EGP$_{supp}$ (%)	59.9 ± 17.4	55.7 ± 11.3	51.6 ± 14.6	0.37	-0.28	-0.30
Circulating Lipids						
Triacylglycerol (mmol·L^{-1})	2.2 ± 0.9	2.2 ± 0.7	1.9 ± 0.7	0.50	Trivial	-0.35
Total Cholesterol (mmol·L^{-1})	4.88 ± 0.59	4.80 ± 0.54	4.62 ± 0.75	0.19	Trivial	-0.26
HDL (mmol·L^{-1})*	0.90 (0.83 – 1.11)	0.88 (0.84 – 1.08)	0.95 (0.88 – 1.18)[b]	**0.01**	Trivial	0.44
LDL (mmol·L^{-1})	2.85 ± 0.61	2.89 ± 0.54	2.77 ± 0.60	0.48	Trivial	-0.21
Hepatokines						
FGF21 (pg·mL^{-1})	176 (110 – 505)	210 (131 – 408)	182 (99 – 463)	0.55	Trivial	Trivial
Follistatin (pg·mL^{-1})	2310 (1534 – 3389)	2104 (1626 – 2683)	2143 (1557 – 2442)	0.37	-0.22	Trivial
Fetuin-A (μg·mL^{-1})	608 ± 138	643 ± 176	614 ± 156	0.67	0.21	Trivial
LECT2 (ng·mL^{-1})	40 ± 5	39 ± 5	39 ± 5	0.51	Trivial	Trivial

Data presented as mean ± SD and for n=9 unless otherwise stated. * indicates that data are not normally distributed and thus presented as median (IQR). [a] the difference between baseline and pre-training values approached statistical significance (uncorrected P-values; FPI P=0.06, HOMA-IR P=0.07, Relative $\dot{V}O_2$ peak P=0.07), [b] significantly different from pre-training values (P<0.05), [c] significantly different from pre-training values (P<0.01), [d] significantly different from pre-training values (P<0.001), [e] the difference between pre-training and post-training values approached statistical significance (uncorrected P=0.09); Trivial effect sizes were considered those of magnitude < 0.2. To convert serum insulin from mU·L^{-1} to pmol·L^{-1}, multiply by 6.

Table 6.2 Habitual sedentary time and physical activity during baseline, pre- and post-training assessment periods

	Baseline	*Pre-training*	*Post-training*	*P-value (main effect of phase)*
General				
Device wear time (minutes per day)	1027 ± 131	989 ± 119	1027 ± 166	0.68
Counts per wear minute	293 ± 111	316 ± 156	294 ± 92	0.34
Sedentary Behaviour				
Minutes per day	719 ± 92	693 ± 139	726 ± 155	0.77
% of wear time[#]	70.3 (64.2 – 74.8)	74.9 (59.6 – 76.6)	66.5 (65.6 – 77.3)	0.37
Light Activity				
Minutes per day	270 ± 74	256 ± 82	262 ± 59	0.60
% of wear time	26.1 ± 5.5	26.0 ± 8.1	25.8 ± 5.5	0.93
Moderate Activity				
Minutes per day[#]	23.0 (18.0 – 50.5)	31.0 (19.5 – 63.0)	30.0 (13.5 – 52.5)	0.89
% of wear time[#]	2.4 (1.6 – 5.5)	2.7 (1.9 – 7.2)	2.5 (1.3 – 6.3)	0.24
Vigorous Activity				
Minutes per day[#]	0.0 (0.0 – 2.0)	0.0 (0.0 – 1.5)	0.0 (0.0 – 1.5)	0.94
% of wear time[#]	0.03 (0.00 – 0.18)	0.01 (0.00 – 0.13)	0.03 (0.00 – 0.13)	0.71
Moderate-Vigorous Activity (MVPA)				
Minutes per day[#]	23.0 (18.5 – 55.0)	31.0 (19.5 – 67.0)	32.0 (13.5 – 59.0)	0.92
% of wear time[#]	2.4 (1.7 – 6.1)	2.7 (1.9 – 7.8)	2.7 (1.3 – 7.1)	0.40

Data presented as mean ± SD, unless otherwise stated. n=9. [#]indicates that data are not normally distributed and thus presented as median (IQR).

P-values represent the main effect of phase.

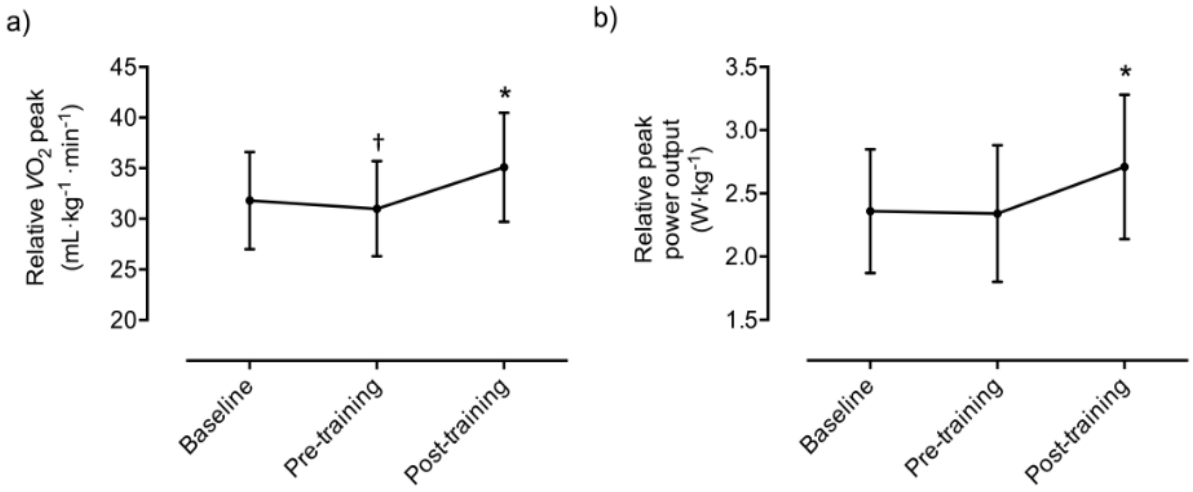


Figure 6.2 a) Peak oxygen uptake ($\dot{V}$O2 peak) and b) peak power output measured at baseline, pre- and post-training. Data presented as mean ± SD (*n*=9). Outcomes presented relative to participant body weight. † indicates the difference between baseline and pre-training values approached statistical significance (unadjusted P = 0.07); * indicates significantly different from pre-training value ($P \leq 0.001$).

6.4.4 Effects of SIT on ectopic fat and systemic metabolic biomarkers

Despite no change in body weight across study visits (main effect of phase: P = 0.17; Table 6.1; Figure 6.3a), SIT elicited a reduction in IHTG (P = 0.03; Figure 6.3b). From pre- to post-training, the mean absolute reduction was 2.1% [-3.4 to 0.8%], which equated to a relative reduction of 12.4% [-31.6 to 6.7%] (g* = -0.23). One MR-image was found to be corrupted at the point of analysis so changes in VAT and ScAT are presented for *n*=8 (characteristics specific to these individuals can be found in Appendix IX). These data do not correspond to the same eight individuals who completed the hyperinsulinaemic, euglycaemic clamp assessments. Training reduced VAT by 16.9% [-24.4 to -9.4%] (g* = -0.62, P = 0.02; Figure 6.3c), but there were no changes in ScAT (main effect; P = 0.16; Figure 6.3d). Training increased circulating HDL by 8.4% [4.6 to 12.2%] (g* = 0.44, P = 0.02;) but total cholesterol, LDL, TG were unchanged throughout the study ($P \geq 0.19$; Table 6.1). Likewise, circulating hepatokine concentrations were similar at baseline, pre- and post-training (≥ 0.37; Table 6.1).

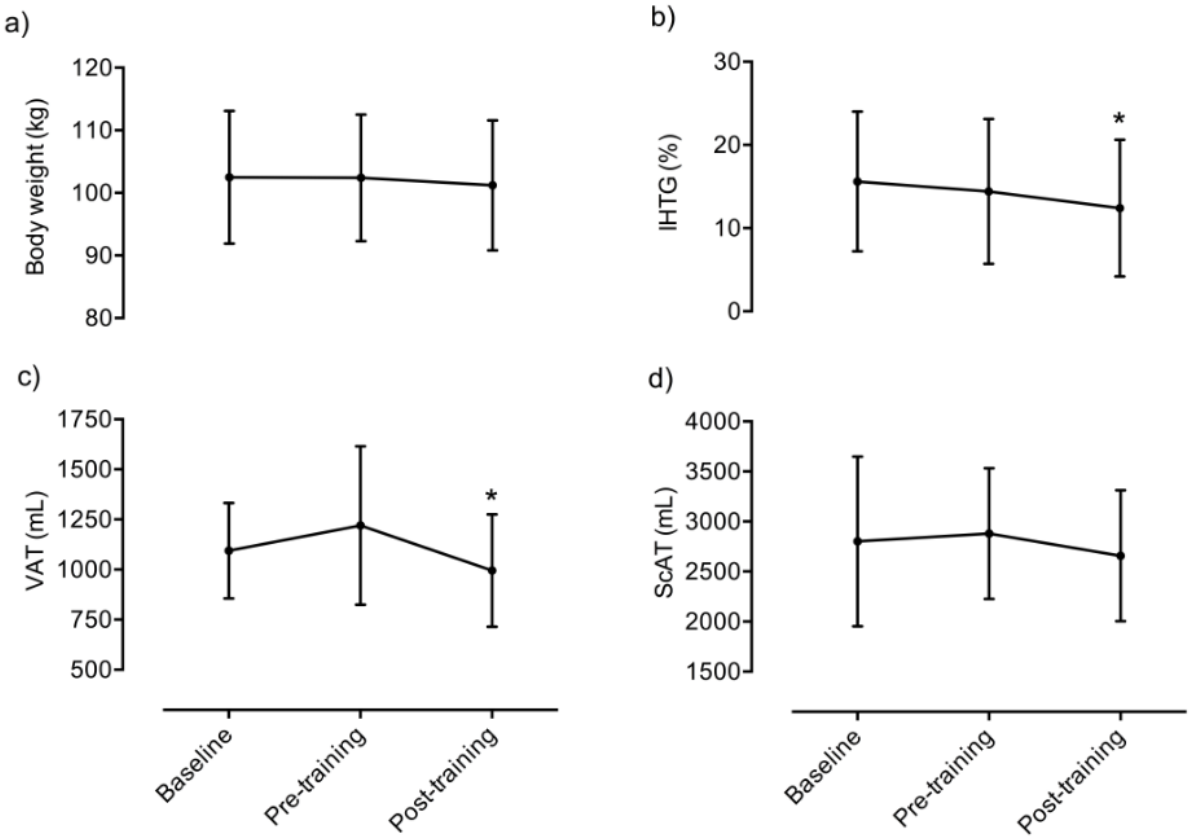


Figure 6.3 a) Body weight, b) intrahepatic triglyceride (IHTG), c) visceral adipose tissue (VAT) and d) subcutaneous abdominal adipose tissue (ScAT) measured at baseline, pre- and post-training. Data presented as mean ± SD. Data for body weight and IHTG are *n*=9. Data for VAT and ScAT are *n*=8. * indicates significantly different from pre-training value ($P \leq 0.03$).

6.4.5 Effects of SIT on peripheral and hepatic insulin sensitivity

Figure 6.4 displays mean circulating insulin concentrations measured throughout dual-step hyperinsulinaemic, euglycaemic clamp assessments. There were no significant differences in the serum insulin concentrations reached at steady state low-dose ($P = 0.55$) or high-dose ($P = 0.64$) insulin infusions between baseline, pre-training and post-training assessments.

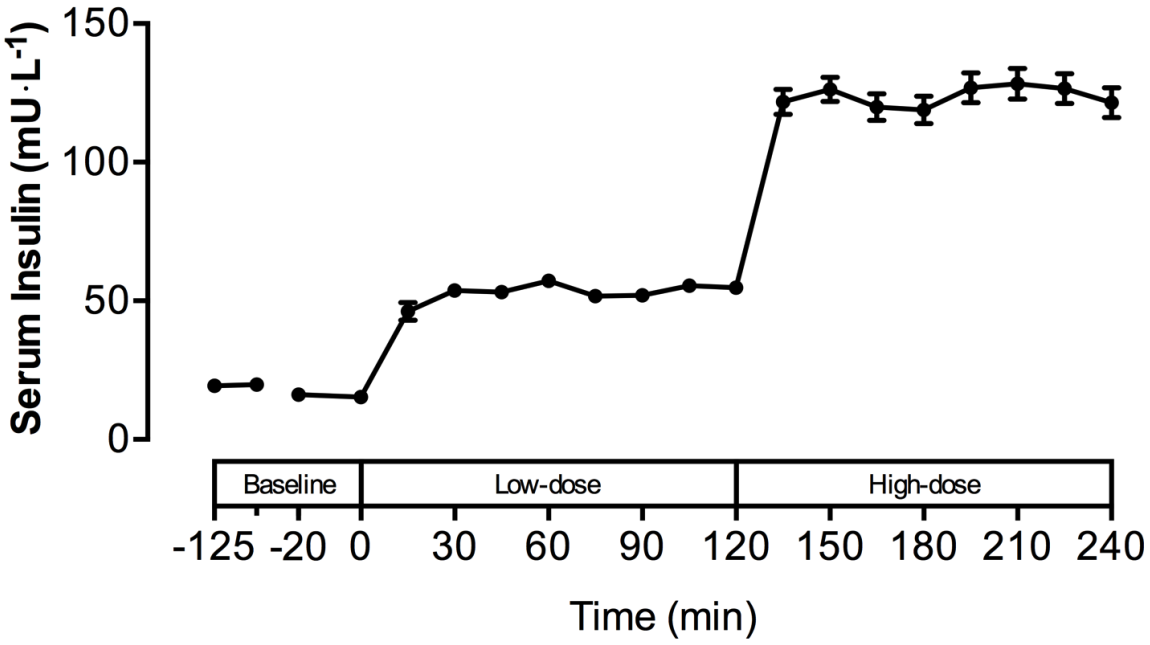


Figure 6.4 Serum insulin concentrations throughout dual-step hyperinsulinaemic, euglycaemic clamp assessments. Data presented as mean ± SD (*n*=8).

There was a significant main effect of phase for the increase in whole-body glucose uptake, which is indicative of peripheral insulin sensitivity, across the three study visits (P = 0.02; Figure 6.5a). However, responses to SIT were highly variable between individuals (range: -18.5% to +58.7%; Figure 6.5b) and, despite a medium effect size (g^* = 0.63), the change from pre- to post-training (18.1% [-3.0 to 39.2%]) was not statistically significant (unadjusted P = 0.09). There was an association between the change in IHTG and the change in whole-body glucose uptake from pre- to post-training (r = -0.83, P = 0.01). EGP in the basal state and during low-dose insulin infusion, HISI (Figure 6.5c) and $\%EGP_{supp}$ (Figure 6.5d), each markers of hepatic insulin sensitivity in the fasted or insulin-stimulated states respectively, did not differ across study visits ($P \geq 0.37$; Table 6.1).

Fasted blood glucose, plasma NEFA and Adipo-IR remained unchanged across the study visits ($P \geq 0.13$; Table 6.1). From pre- to post-training, fasted serum insulin (-13.9% [-24.9 to -2.9%], g^* = -0.48, P = 0.04) and HOMA-IR (-16.6% [-27.5 to -5.6%], g^* = -0.56, P = 0.02) were reduced. However, these reductions were similar in magnitude to the increases from baseline to pre-training (unadjusted P = 0.06 and P = 0.07, respectively) such that post-training values were no different from those measured at baseline ($P \geq 0.68$; Table 6.1).

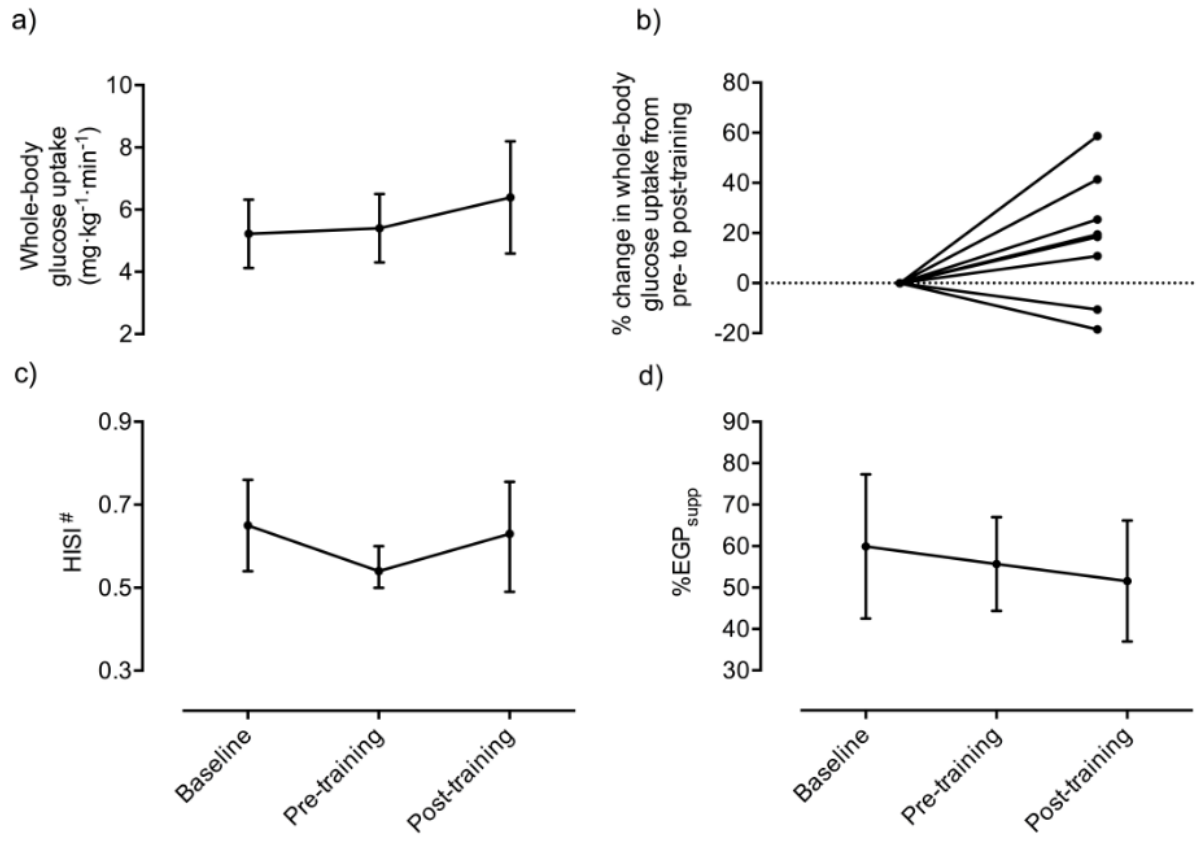


Figure 6.5 a-b) Peripheral and c) basal and d) insulin-stimulated hepatic insulin sensitivity measured at baseline, pre- and post-training. Data in 'A' and 'D' presented as mean ± SD (*n*=8). #Data in 'C' are not normally distributed and thus presented as median (IQR). Data in 'B' are % change from pre- to post-training measurements for each participant.

6.5 Discussion

The principal findings of this study are that a six-week SIT intervention is feasible for individuals with NAFLD and is well adhered to in a controlled laboratory setting. Furthermore, six weeks of SIT reduces IHTG and VAT in the absence of body weight change and, whilst hepatic insulin sensitivity appears to be unaffected, changes in peripheral insulin sensitivity are highly variable between individuals.

This study reports almost perfect adherence to a six-week SIT intervention in nine individuals. Specifically, every participant completed the exercise programme, attending all 18 training sessions. Eight participants completed all 90 of the prescribed intervals whilst the remaining participant completed 87 intervals, with the three missing intervals contained within the first two training sessions. The implication is that individuals with NAFLD are able and willing to perform exercise training sessions composed of bursts of maximal exercise. This is important because observational evidence (Kistler *et al.*, 2011) and experimental data (Cho *et al.*, 2015; Oh *et al.*, 2017) suggest that high-intensity exercise may be more potent in attenuating IHTG accumulation and NAFLD progression than moderate-intensity exercise. This SIT intervention may, therefore, represent a model of exercise that facilitates the completion of more intense exercise in individuals with NAFLD. This intervention was performed in a tightly controlled laboratory setting, with specialist equipment and where participants were individually supported by the research team. The participants recruited to this study were also screened thoroughly to ensure the absence of advanced cardiometabolic disease and may, therefore, not be representative of the majority of individuals with NAFLD. Given that the risk of an acute cardiac event during exercise is elevated in previously inactive individuals with established cardiometabolic disease (Thompson *et al.*, 2007), the implementation of SIT requires additional scrutiny. The necessity for medical clearance and acclimatisation to exercise must be considered in this context (Riebe *et al.*, 2015).

This study reports a mean absolute reduction in IHTG of 2.1% after six weeks of SIT in individuals with elevated IHTG (relative change from baseline: -12.4%). Previous studies employing aerobic and resistance exercise, combined or in isolation, report a reduction of similar magnitude (10 to 21%) in the absence of significant weight loss (Johnson *et al.*, 2009; Hallsworth *et al.*, 2011; Sullivan *et al.*, 2012; Keating *et al.*, 2015; Pugh *et al.*, 2016; Houghton *et al.*, 2017). However, greater reductions in IHTG (27 to 42%) have been reported when significant weight loss occurs as a result of exercise training (Hallsworth *et al.*, 2015; Keating

et al., 2015; Cuthbertson *et al.*, 2016; Zhang *et al.*, 2016). Therefore, whilst the independent effects of exercise on IHTG are recognised (Brouwers *et al.*, 2016), the greatest benefits occur when exercise contributes to a negative energy balance.

It was beyond the scope of this study to examine the mechanisms through which SIT reduced IHTG. However, some speculative inferences can be made. There was a small reduction in mean body weight from pre- to post-SIT (mean 1.2 kg) which, whilst not statistically significant, may have impacted on the reduction in IHTG reported. Using the established, albeit simplified, rule of thumb that a 3500 kcal energy deficit corresponds to an approximate 1 lb loss of fat mass (Guth, 2014), and assuming that substantial changes in lean body mass over the 6 week SIT intervention are unlikely, the 1.2 kg (2.65 lbs) weight loss reported in this study corresponds to a total energy deficit of approximately 9275 kcal. Previous studies have reported that the energy expenditure of a single SIT session is approximately 143 kcal (Deighton et al., 2013). Therefore, the total energy expenditure that can be attributed to our intervention (18 sessions) is approximately 2574 kcal. Consequently, the intervention alone does not account for the weight loss reported in this study. Habitual physical activity was consistent across the duration of our study, but it may be that changes in energy intake or other components of daily energy balance (such basal metabolic rate) may have occurred. The exact nature of these potential changes is unclear at present, with the exiting literature reporting large heterogeneity in compensatory responses to SIT at both the individual and study level (Taylor et al., 2018; Schubert et al., 2017).

In addition to the small degree of weight loss reported, metabolic factors may also underpin the reported change in IHTG (Brouwers *et al.*, 2016). Neither fasted circulating NEFA nor Adipo-IR differed throughout the current study, suggesting that an improvement in adipose tissue insulin sensitivity in the fasted state is unlikely to be responsible for the reduction in IHTG with training. However, the possibility that changes in postprandial adipose tissue insulin sensitivity occurred cannot be dismissed (Brouwers *et al.,* 2016). Circulating glucose stimulates hepatic *de novo* lipogenesis (Ameer *et al*., 2014) and, although fasted blood glucose was unchanged throughout the study, post-prandial glucose is likely to have been reduced in those with improved peripheral insulin sensitivity. Therefore, particularly in individuals who displayed improvements in whole-body glucose uptake, a reduction in hepatic *de novo* lipogenesis may have contributed to post-training reductions in IHTG (Linden *et al*., 2015). Lastly, altered very low-density lipoprotein metabolism is unlikely to be responsible for

exercise-induced reductions in IHTG (Sullivan *et al.,* 2012) but enhanced capacity to oxidise hepatic lipid is possible (Linden *et al.,* 2015).

Peripheral insulin sensitivity increased throughout this study but individual changes from pre- to post-SIT were highly variable and, despite a mean relative increase of 18.1% and a moderate effect size, this change was not statistically significant. In obese but otherwise healthy men, four weeks (Cocks *et al.*, 2015), but not two weeks (Whyte, Gill and Cathcart, 2010), of SIT increased peripheral insulin sensitivity. However, 15 to 20% of individuals may display minimal, or even adverse, responses after exercise training in outcomes related to glucose homeostasis (Stephens and Sparks, 2015) and insulin sensitivity improved in only 10 out of 12 healthy individuals who completed a two-week SIT intervention, remaining unchanged in one and decreasing in another (Richards *et al.*, 2010). This degree of variation in response to exercise is consistent with our findings where peripheral insulin sensitivity improved in 75% of participants after SIT, yet was reduced in 25%. Given this variation, a greater sample size may be required to detect significant differences in peripheral insulin sensitivity following SIT. A retrospective sample size calculation indicates that, with the current change in whole-body glucose uptake (mean ± SD = 0.99 ± 1.41 $mg \cdot kg^{-1} \cdot min^{-1}$; thus standardised difference = 0.70; within-person correlation = 0.63), 14 individuals would have been required to detect a statistically significant difference from pre- to post-training with 80% power and an alpha error rate of 0.05. A number of factors, including genetic polymorphisms, epigenetics and baseline participant characteristics, may impact on individual responses (Böhm *et al.*, 2016).

Neither basal nor insulin-stimulated hepatic insulin sensitivity are changed after six weeks of SIT. Our findings agree with data showing no change in hepatic insulin sensitivity after 12 weeks of aerobic training in patients with NAFLD, despite significant reductions in body weight (Cuthbertson *et al.*, 2016). EGP during low-dose insulin infusion is reduced after aerobic or combined aerobic-plus-resistance exercise training in sedentary, healthy individuals and in patients with T2DM (Shojaee-Moradie *et al.*, 2007; Meex *et al.*, 2010). However, neither of these studies report changes in basal EGP or HISI, and the change at low-dose insulin infusion reported by Meex *et al.* was no longer statistically significant when presented as $\%EGP_{supp}$. EGP in both the basal state and during low-dose insulin infusion was reduced in overweight, older women after a 9-month moderate-intensity aerobic exercise intervention (DiPietro *et al.*, 2006). However, this may have been due to notably higher rates of EGP in this group at baseline compared to both the control group, and a separate group completing a higher-intensity exercise programme. The high-intensity exercise training had no effect on EGP in

either the basal or insulin-stimulated states (DiPietro *et al.*, 2006). The lack of improvements in hepatic insulin sensitivity in the current study may be due to insufficient intervention duration or because IHTG at the end of the intervention remained elevated (Cuthbertson *et al.*, 2016). Hepatic insulin sensitivity, assessed as $\%EGP_{supp}$, may be impaired with as little as 1.5% IHTG, with no further deterioration as IHTG increases (Bril, Barb, *et al.*, 2017). Post-training IHTG values in the present study ranged from 4.3 to 25.9% (mean 12.4%).

The favourable changes in IHTG, VAT, HDL and cardiorespiratory fitness in response to SIT are important for individuals with NAFLD. NAFLD is intricately related to the metabolic syndrome and associated with an elevated risk of T2DM, cardiovascular and renal disease (Byrne and Targher, 2015). Ectopic lipid and dyslipidaemia are components of the metabolic syndrome and improvements in these risk factors are important in the treatment of NAFLD. Additionally, cardiorespiratory fitness is a marker of metabolic health and inversely associated with all-cause and cardiovascular mortality (Blair *et al.*, 1989; Kodama *et al.*, 2009). The large increase following SIT most likely reflects metabolic adaptations within skeletal muscle (Gibala *et al.*, 2012), which may provide benefit via improved substrate metabolism (Rabøl *et al.*, 2011; Brouwers *et al.*, 2016). Collectively, the present study demonstrates the potential of SIT to elicit relevant metabolic improvements in men with NAFLD, but it is notable that the magnitude of response over this intervention was insufficient to re-establish values in an optimal range. Furthermore, we report no effect of SIT on other cardiovascular risk factors, such as circulating TG, total cholesterol and LDL. Each of these have been previously shown to be reduced by exercise training (Garber et al., 2011). Whilst our null findings may be the result of a lack of statistical power, we report a small effect size from pre- to post-SIT for each of these outcomes (g^* = -0.21 to -0.35). Alternatively, it may be that the intervention was insufficient in duration or that more traditional forms of exercise training, such as CME, which are associated with greater energy expenditure and weight loss, may be required to elicit potent benefits on these outcomes.

It should also be noted that we saw no significant change in circulating hepatokines as a result of our SIT intervention and the effect size for each hepatokine measured was trivial. As stated previously, however, changes in body weight, IHTG and indices of metabolic health were also small in this study. It may be larger changes in metabolic parameters than those seen in the current study are required to modulate circulating hepatokine concentrations.

A strength of this study is the use of the most precise techniques available to assess key outcomes. Conversely, this study was conducted in a relatively small and homogenous sample of white European men with no other chronic metabolic disease. We may have lacked statistical power to detect differences in some of our outcomes following training and null-findings may be the result of type II error. The findings of this study may also not be generalisable to women, individuals of different ethnicity or those with metabolic co-morbidities. Furthermore, participants did not have a formal diagnosis of NAFLD so we have no information regarding disease severity. An RCT design would also have been preferred. However, the inclusion of a control phase within our within-measures study design was chosen to monitor variation in study outcomes over a period of no intervention, whilst avoiding the additional recruitment of a non-exercise control group.

In this study we have shown that men with NAFLD are compliant with SIT which, over six weeks, improves cardiorespiratory fitness and reduces IHTG and VAT, without altering body weight. Furthermore, changes in peripheral insulin sensitivity with training are highly variable between individuals, whilst hepatic insulin sensitivity remains unchanged. These results support the potential for interval-based, high-intensity exercise as an alternative to continuous moderate-intensity exercise in the management of NAFLD. However, larger RCTs are required to test the effectiveness of SIT in diverse populations, as well as its applicability in a clinical setting, sustainability over time and efficacy in individuals with advanced NAFLD.

CHAPTER 7

THE EFFECT OF EXERCISE TRAINING ON INTRAHEPATIC TRIGLYCERIDE AND HEPATIC INSULIN SENSITIVITY: A SYSTEMATIC REVIEW AND META-ANALYSIS

This study presented in this chapter has been published and has the following citation:

Sargeant JA, Gray LJ, Bodicoat DH *et al*. (2018). *Obesity Reviews.* **19**: 1446-1459.

This chapter presents the published manuscript.

7.1 Abstract

This systematic review and meta-analysis determined the impact of structured exercise training, and the influence of associated weight loss, on intrahepatic triglyceride (IHTG) in individuals with non-alcoholic fatty liver disease (NAFLD). It also examined its effect on hepatic insulin sensitivity in individuals with or at increased risk of NAFLD. Analyses were restricted to studies using magnetic resonance spectroscopy or liver biopsy for the measurement of IHTG and isotope-labelled glucose tracer for assessment of hepatic insulin sensitivity. Pooling data from 17 studies (373 exercising participants), exercise training for one to 24 weeks (mode: 12 weeks) elicits an absolute reduction in IHTG of 3.31% (95% CI: -4.41 to -2.22%). Exercise reduces IHTG independent of significant weight change (-2.16 [-2.87 to -1.44]%), but benefits are substantially greater when weight loss occurs (-4.87 [-6.64 to -3.11]%). Furthermore, meta-regression identified a positive association between percentage weight loss and absolute reduction in IHTG (β = 0.99 [0.62 to 1.36], P<0.001). Pooling of six studies (94 participants) suggests that exercise training also improves basal hepatic insulin sensitivity (mean change in hepatic insulin sensitivity index: 0.13 [0.05 to 0.21] $mg \cdot m^{-2} \cdot min^{-1}$ per $\mu U \cdot mL^{-1}$), but available evidence is limited and the impact of exercise on insulin-stimulated hepatic insulin sensitivity remains unclear.

7.2 Introduction

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease worldwide (Younossi *et al.*, 2016) and a prominent risk factor for cardiovascular disease, chronic kidney disease and type 2 diabetes mellitus (T2DM) (Byrne and Targher, 2015). Insulin resistance promotes hepatic lipid accumulation, which is most commonly assessed via the measurement of intrahepatic triglyceride (IHTG) (Samuel *et al.*, 2004; Taylor, 2008; Kumashiro *et al.*, 2011; Shulman, 2014). The associated accumulation of lipid intermediates, such as diacylglycerol (DAG), may in turn perpetuate insulin resistance (Samuel *et al.*, 2004; Taylor, 2008; Kumashiro *et al.*, 2011; Shulman, 2014); providing a mechanistic link between NAFLD and impaired metabolic regulation. As such, strong associations exist between excess IHTG and insulin resistance in multiple tissues, including the liver (Korenblat *et al.*, 2008; Shulman, 2014; Bril, Barb, *et al.*, 2017). Defects in hepatic insulin signalling contribute to elevated endogenous glucose production (EGP) which is integral to the pathophysiology of impaired glucose regulation and T2DM (Taylor, 2008; Rizza, 2010; Petersen, Vatner and Shulman, 2017).

Prompted by reports that exercise training has the capacity to reduce IHTG in the absence of weight loss (Johnson *et al.*, 2009; Sullivan *et al.*, 2012), the independent effects of exercise in the treatment of NAFLD have been examined (Keating *et al.*, 2012; Orci *et al.*, 2016; Smart *et al.*, 2016; Hashida *et al.*, 2017; Katsagoni *et al.*, 2017). These reviews confirm the ability of exercise to reduce IHTG without significant weight change; however, the importance of the exercise-related energy deficit and subsequent weight loss has not been investigated thoroughly. Acute and sustained energy restriction and weight loss potently reduces IHTG in individuals with NAFLD (Kirk *et al.*, 2009; Hickman *et al.*, 2013) and therefore logic dictates that weight loss associated with exercise training would be an important mediator of the IHTG response to exercise training. This issue has practical implications for the prescription of exercise in the management of NAFLD and thus deserves explicit attention.

Previous reviews highlight a range of different methods to estimate IHTG, including non-invasive imaging by ultrasound (US) or computed tomography (CT), proton magnetic resonance spectroscopy (^{1}H-MRS) and invasive liver biopsy. The inclusion of multiple methods within these reviews has the benefit of broadening study eligibility and thus increasing pooled participant sample size. However, it also adds an additional source of heterogeneity. US and CT are also limited by a lack of sensitivity to detect mild-to-moderate accumulation of

IHTG and to quantify subtle changes resulting from experimental interventions (Festi *et al.*, 2013). ^{1}H-MRS has much greater precision (Bawden, Scott and Aithal, 2017), making it a more suitable method for experimental research, whilst the necessity to characterise histological features beyond steatosis make liver biopsy the standard tool in clinical practice (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018).

The effect of exercise training on hepatic insulin sensitivity has not been reviewed. A number of indices exist which assess insulin resistance using simple circulating biomarkers (Matsuda and DeFronzo, 1999) but stable or radioactive isotope-labelled tracers are required to obtain the most accurate measurement of insulin sensitivity in individual tissues (Kim *et al.*, 2016). Glucose tracers can be used to quantify EGP (primarily attributed to hepatic glucose production) (Petersen, Vatner and Shulman, 2017) to accurately assess hepatic insulin sensitivity in the basal (fasted) and insulin-stimulated (post-prandial) states; using the hepatic insulin sensitivity index (HISI) and percentage suppression of EGP ($\%EGP_{supp}$) by low-dose insulin infusion, respectively (DeFronzo, Simonson and Ferrannini, 1982; Matsuda and DeFronzo, 1999).

This systematic review and meta-analysis had two primary aims. First, we investigated the effects of structured exercise training on IHTG in individuals with NAFLD, with a particular focus on the impact of concurrent weight loss, whilst restricting analyses to studies using ^{1}H-MRS and liver biopsy. Second, we explored the effects of exercise training on basal and insulin-stimulated hepatic insulin sensitivity.

7.3 Methods

The current systematic review and meta-analysis (PROSPERO ID: CRD42014007268) was conducted in accordance with the "Cochrane handbook for systematic reviews of interventions" and PRISMA guidelines (Moher *et al.*, 2009; Higgins and Green, 2011). All aspects of the literature search, study selection and risk of bias assessment were completed by two researchers independently (Jack Sargeant and James King/Scott Willis). Data extraction and analysis were performed by a single researcher (Jack Sargeant) before being checked, independently, by another (extraction: James King; analysis: Danielle Bodicoat/Laura Gray).

7.3.1 Primary outcomes

This review had two outcome measures:

- IHTG
- Hepatic insulin sensitivity (basal and insulin-stimulated)

Eligible studies were restricted to those using ^{1}H-MRS or liver biopsy for the measurement of IHTG and using isotope-labelled glucose tracer to quantify EGP in the fasted state and following low-dose ($\leq$ 20 mU·m^{-2}·min^{-1} or $\leq$ 0.5 mU·kg^{-1}·min^{-1}) insulin infusion, for the calculation of HISI and %EGP$_{supp}$, respectively.

7.3.2 Literature search

Six electronic online databases (EMBASE, MEDLINE, PubMed, Scopus, Sport Discus and Web of Science) were searched from inception to July 2017 using the following terms related to exercise, IHTG and hepatic insulin sensitivity (all terms within brackets were combined using 'OR'):

> (exercise, physical activity, training) AND [(liver fat, hepatic fat, intrahepatic triglyceride, IHTG, intrahepatocellular lipids, intrahepatic lipids, non-alcoholic fatty liver disease, NAFLD, non-alcoholic fatty liver, fatty liver, hepatic steatosis, non-alcoholic steatosis, liver steatosis, non-alcoholic steatohepatitis, NASH, hepatic steatohepatitis, liver steatohepatitis, liver function) OR (hepatic insulin sensitivity, hepatic insulin resistance, liver insulin sensitivity, liver insulin resistance, hepatic IR, liver IR, hepatic glucose production, liver glucose production, endogenous glucose production, glycolysis, gluconeogenesis)].

Reference lists of all included manuscripts were screened for further eligible studies.

7.3.3 Study selection

Human experimental studies written in the English language were included. Conference abstracts were considered but underwent the same eligibility and risk of bias assessments as full articles. Eligible studies were those in which participants with overweight or obesity completed an exercise training programme of at least three exercise sessions. All types of study design were considered. Studies investigating changes in IHTG were only eligible if participants had diagnosed NAFLD or where baseline characteristics met diagnostic criteria (IHTG > 5% in the absence of secondary steatogenic sources, including excessive alcohol intake and viral infection) (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018). NAFLD was not an inclusion criterion for studies investigating changes in hepatic insulin sensitivity as it became apparent that very few studies have examined this outcome exclusively in this patient group and several otherwise eligible studies did not measure IHTG. Studies investigating exercise in combination with dietary intervention were eligible only when data were available for matched, independent groups prescribed exercise training with or without diet. In studies measuring hepatic insulin sensitivity, it was essential that participants refrained from strenuous exercise for at least 48 hours before assessments (Sylow *et al.*, 2017).

7.3.4 Data extraction

Descriptive information (first author and year of publication), details of study design, participant and intervention characteristics and outcome data were extracted from eligible manuscripts. Outcome data were extracted as mean change and standard deviation from pre- to post-intervention for all exercise groups, as well as for the control groups of randomised controlled trials (RCTs). Where possible, outcome data presented in alternative forms were converted as outlined in Appendix X. When data were presented in graphical form only, values were estimated using commercially available software (Digitizeit, Version 2.2, Bormann, I., Braunschweig, Germany). When incomplete or insufficient data were reported, the authors were contacted and if the required data were unavailable the study was removed. When characteristics of exercise interventions (such as the frequency, intensity or duration of exercise sessions) progressed over the course of a programme, a weighted mean was calculated. Further details of data extraction can be found in Appendix X.

A number of variations of HISI exist, including the statistical inverse (the hepatic insulin resistance index; HIRI). Studies reporting these alternatives were included in qualitative review

but were only included in quantitative meta-analysis when raw data were available for the calculation of HISI as originally described (Matsuda and DeFronzo, 1999):

1000 / (EGP [$mg \cdot m^{-2} \cdot min^{-1}$] x fasted plasma insulin [$\mu U \cdot mL^{-1}$])

Studies that reported EGP and fasted plasma insulin (FPI) separately were also considered, but were excluded from all analyses unless raw data were available for the calculation of HISI as above. Mean values of EGP and FPI were not combined. Similarly, when study design allowed the calculation of $\%EGP_{supp}$ but it was not reported, raw data were requested.

7.3.5 Risk of bias assessment

Studies were assessed for risk of bias using a modified Downs and Black scale (Downs and Black, 1998). This checklist includes 26 items divided into categories of (i) reporting, (ii) external validity, (iii) internal validity – bias, (iv) internal validity – confounding and (v) power. The Downs and Black scale was modified in two ways. Blinding participants and experimenters to group allocations is a difficult task in exercise trials. It is possible, however, to blind experimenters who are conducting data analysis. As such, item 14 of the original scale (concerning participant blinding) was removed, whilst item 15 (concerning experimenter blinding) was scored according to whether attempts were made to blind experimenters during data analysis. Item 27, concerning statistical power, was modified as follows:

Formal power calculation performed based on detecting a significant change in IHTG = score of two awarded

Formal power calculation performed based on detecting a significant difference in a relevant and related outcome but which was not IHTG = score of one awarded

No formal power calculation performed = score of zero awarded.

Consensus between the two independent assessors (Jack Sargeant/James King) was ensured. Publication bias was assessed using funnel plots.

7.3.6 Meta-analyses

Pooled characteristics of study participants are presented as weighted means, accounting for differences in sample size, along with the range. Quantitative analysis was conducted using commercially available software (Stata IC, Version 14.1, StataCorp LP, Texas, USA). Pooled mean differences with 95% confidence intervals (95% CI) were calculated for primary

outcomes using random effects models and heterogeneity was assessed quantitatively using the I^2 statistic.

Where possible, primary outcomes were analysed in two ways:

- *Within-group analysis*: The change from pre- to post-intervention measurements in all exercise groups of all eligible studies.
- *Between-group analysis*: The difference in the change from pre- to post-intervention between exercise and control groups in RCTs only.

In RCTs with multiple intervention groups, the intervention groups were combined, where possible, using appropriate statistical formulae (Higgins and Green, 2011) and, for the purpose of exercise programme description, the exercise intensity, duration and frequency of the combined group were calculated as the weighted mean of the individual groups. When groups were not suitable to be combined (for example, aerobic and resistance exercise training groups), data from the aerobic intervention group was used.

7.3.7 Subgroup analyses and meta-regression

Subgroup analyses were performed to investigate whether the presence of significant weight loss (defined as a statistically significant reduction in body weight from pre- to post-intervention) explained heterogeneity in the response of IHTG to exercise training. The influence of the exercise mode (aerobic, high-intensity interval (HIIT), resistance or combined aerobic/HIIT-plus-resistance training) was also investigated, as was the exercise intensity (moderate- or high-intensity) of aerobic and HIIT interventions. Exercise intensity was categorised according to published criteria (Garber *et al.*, 2011), which are summarised in Table 7.1. One study (Meex *et al.*, 2010) prescribed exercise relative to maximal workload. This study was categorised using the same percentage categories as those of $\dot{V}O_2$ peak. Meta-regression was also performed to explore the effects of intervention duration and the magnitude of body weight change on changes in IHTG. All subgroup analyses and meta-regressions were performed using the mean change from pre- to post-training in all exercise groups of eligible studies. Secondary analyses of hepatic insulin sensitivity were not performed due to the limited number of studies identified.

Table 7.1 Categories of exercise intensity

	RPE	**%HR max**	**%HRR**	**%$\dot{V}O_2$ peak**
Moderate-intensity	12 – 13	64 – 76	40 – 59	46 – 63
High-intensity	14 – 17	77 – 95	60 – 89	64 – 90

Adapted from (Garber *et al.*, 2011); **%**HR max: percentage of maximal heart rate; %HRR: percentage of heart rate reserve; %$\dot{V}O_2$ peak: percentage of peak oxygen uptake; RPE: rating of perceived exertion (Borg, 1970).

7.4 Results

7.4.1 Literature search

Figure 7.1 presents a flowchart of the literature search and study selection processes. To summarise, 20,055 records were returned by the six online databases, along with five from reference lists of eligible manuscripts. Of these, 111 manuscripts underwent full assessment and 21 were eligible for inclusion (20 for meta-analyses). Twenty were complete articles and one was a conference abstract, although the latter has since been published in full (Sargeant, Bawden, *et al.*, 2018). Table 7.2 presents an overview of all eligible studies and readers are directed to Appendix XI for more detailed description of participant and intervention characteristics for each study.

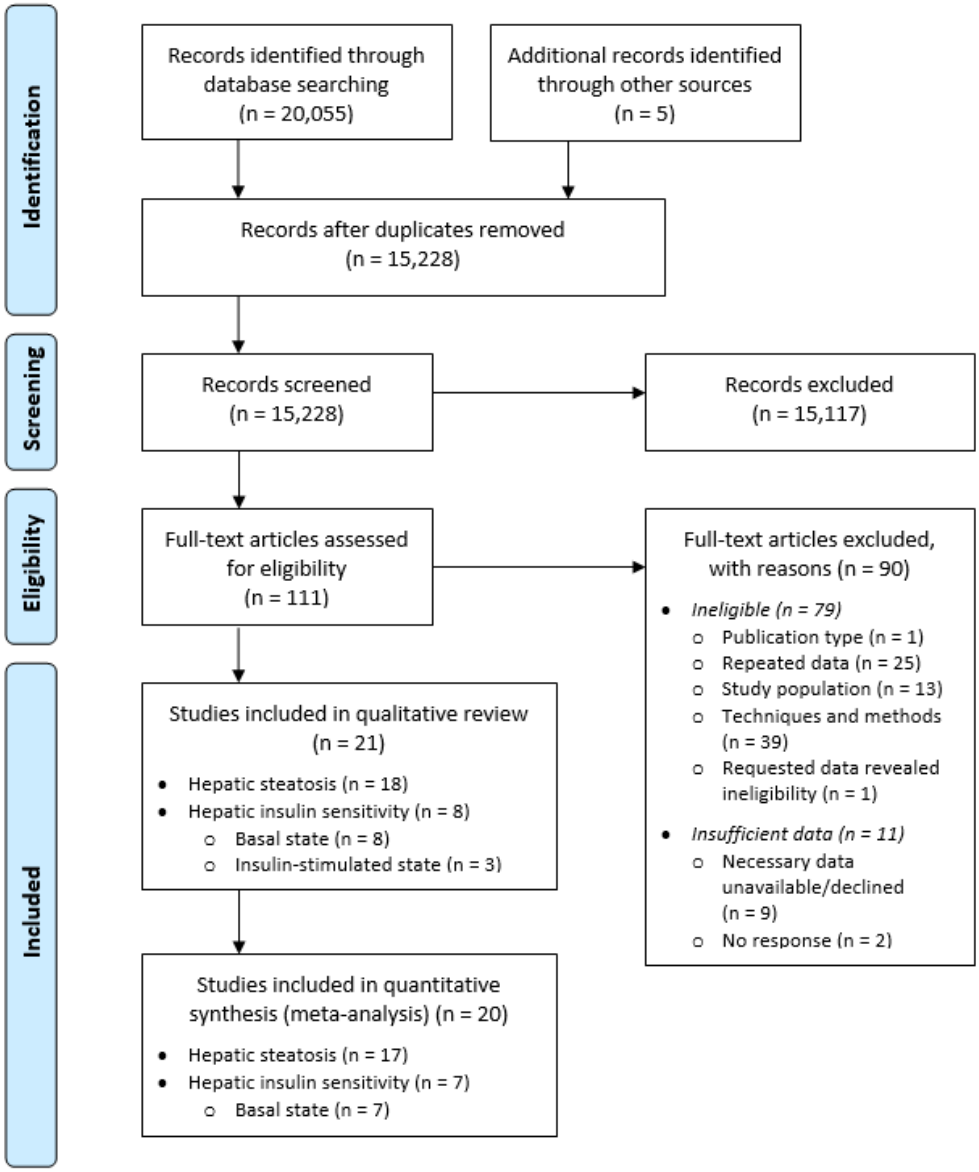


Figure 7.1 Flowchart of literature search process

Table 7.2 Overview of included studies (continued overleaf)

First Author (Year of Publication)	Study Design	Sample Size (M/F)	Exercise Mode	Intervention Duration	Session Frequency	Exercise Intensity (Category)	Technique used for Assessment of IHTG	Index of Basal Hepatic IS Originally Reported	Details of Low-Dose Insulin Infusion	Weight Change with Exercise Training (mean % change from baseline)
Cassidy (2016)	RCT	Ex: 12 (10/2) Con: 11 (8/3)	HIIT	12 weeks	3 times per week	High	^{1}H-MRS	-	-	-1.1 $^{\beta}$
Cuthbertson (2016)	RCT	Ex: 30 (23/7) [12 (8/3/1 NR)] Con: 20 (16/4) [7 (3/4)]	Aerobic	16 weeks	3 – 5 times per week	Moderate	^{1}H-MRS	HIRI	0.3 mU•kg^{-1}•min^{-1} for 120 min	-2.5 $^{\alpha, \beta}$
Hallsworth (2011)	RCT	Ex: 11 (NR) Con: 8 (NR)	Resistance	8 weeks	3 times per week	N/A	^{1}H-MRS	-	-	0.0
Hallsworth (2015)	RCT	Ex: 12 (6/6) Con: 11 (10/1)	HIIT	12 weeks	3 times per week	High	^{1}H-MRS	-	-	-1.6 $^{\alpha, \beta}$
Haus (2013)	Uncontrolled Intervention	Ex: 17 (NR)	Aerobic	1 week	7 times per week	High	^{1}H-MRS	-	-	0.2
Hickman (2013)	RCT*	Ex: 9 (7/2) [13 (9/4)]	Resistance	24 weeks	3 times per week	N/A	Biopsy†	HIRI	-	-2.6
Houghton (2017)	RCT	Ex: 12 (7/5) Con: 12 (7/5)	Combined (HIIT + Resistance)	12 weeks	3 times per week	High	^{1}H-MRS	-	-	1.1
Johnson (2009)	RCT	Ex: 12 (NR) Con: 7 (NR)	Aerobic	4 weeks	3 times per week	Moderate	^{1}H-MRS	-	-	-0.3

Keating (2015)	RCT	Ex1: 12 (6/6) Ex2: 11 (5/6) Con: 12 (3/9)	Aerobic	8 weeks	Ex1: 3 sessions per week Ex2: 4 sessions per week	Ex1: High Ex2: Moderate	^{1}H-MRS	-	-	Ex1: -1.2 $^{\alpha, \beta}$ Ex2: -1.5 $^{\alpha, \beta}$
Langleite (2016)	Uncontrolled Intervention	Ex: 11 (11/0)	Combined (Aerobic + HIIT + Resistance)	12 weeks	4 times per week (1 aerobic, 1 HIIT, 2 resistance)	High	^{1}H-MRS	-	-	-1.2
Lee (2013)	RCT	Ex1: [16 (0/16)] Ex2: [16 (0/16)] Con: [12 (0/12)]	Ex1: Aerobic Ex2: Resistance	12 weeks	3 times per week	High	-	HISI†	-	Ex1: -1.3 Ex2: -0.3
Malin (2013)	Uncontrolled Intervention	Ex: 13 (6/7)	Aerobic	1 week	7 times per week	High	^{1}H-MRS	-	-	0.6
Meex (2010)	Uncontrolled Intervention	Ex1: [20 (20/0)] Ex2: [17 (17/0)]	Combined (Aerobic + Resistance)	12 weeks	3 times per week (2 aerobic, 1 resistance)	Moderate	-	HISI$^{\#}$	-	Ex1: -1.1 Ex2: -1.0
Oh (2014)	Uncontrolled Intervention	Ex: 18 (4/14)	Vibration / Acceleration	6 weeks	3 times per week	N/A	^{1}H-MRS	-	-	-0.4 $^{\alpha}$
Pugh (2014)	RCT	Ex: 13 (7/6) Con: 8 (4/4)	Aerobic	16 weeks	3 – 5 times per week	Moderate	^{1}H-MRS	-	-	-2.4
Sargeant (2018)	Controlled Longitudinal Intervention	Ex: 9 (9/0) [8 (8/0)]	HIIT	6 weeks	3 times per week	High	^{1}H-MRS	HISI	20 mU•m^{-2}•min^{-1} for 120 min	-1.2
Shojaee-Moradie (2007)	RCT	Ex: [10 (10/0)] Con: [7 (7/0)]	Aerobic	6 weeks	3 times per week	High	-	HISI$^{\#}$	0.3 mU•kg^{-1}•min^{-1} for 120 min	-0.8

Sullivan (2012)	RCT	Ex: 12 (4/8) Con: 6 (1/5)	Aerobic	16 weeks	5 times per week	Moderate	^{1}H-MRS	-	-	-0.2 $^{\alpha}$
van der Heijden (2010a)‡	Uncontrolled Intervention	Ex: 15 (7/8) [15 (7/8)]	Aerobic	12 weeks	4 times per week	High	^{1}H-MRS	HISI	-	-0.5
van der Heijden (2010b)	Uncontrolled Intervention	Ex: 7 (NR) [12 (6/6)]	Resistance	12 weeks	2 times per week	N/A	^{1}H-MRS	HISI	-	2.6 $^{\alpha}$
Zhang (2016)	RCT	Ex1: 73 (22/51) Ex2: 73 (21/52) Con: 74 (28/46)	Aerobic	24 weeks	5 times per week	Ex1: Moderate Ex2: High	^{1}H-MRS	-	-	Ex1: -2.8 $^{\alpha, \beta}$ Ex2: -6.0 $^{\alpha \beta}$

Sample sizes in squared brackets represent the number of participants included in hepatic insulin sensitivity outcomes; Mean changes in body weight with exercise are as reported in the original manuscript ($^{\alpha}$ indicates significantly different from baseline, $^{\beta}$ indicates significantly different from non-exercise control group); Exercise intensity is categorised according to published criteria (Garber *et al.*, 2011), which are summarised in Table 7.1; *Study did not include a 'standard care' or 'no intervention' group, exercise was compared with hypocaloric diet; †Study was excluded from one or more meta-analyses - Hickman *et al* (2013) was removed from meta-analyses of changes in hepatic steatosis to reduce heterogeneity as it was the only study using liver biopsy to assess IHTG. Lee *et al* (2013) was removed from meta-analysis of changes in basal hepatic insulin sensitivity because raw data were not available to allow re-calculation of HISI as outlined by Matsuda and DeFronzo (1999); $^{\#}$Published manuscript contained no index of hepatic insulin sensitivity. HISI was calculated after authors provided raw data; ‡Manuscript refers to the same study as van der Heijden *et al* (2009); Con: Control group; Ex1/2: Exercise group 1/2; N/A: not applicable; NR: not reported.

7.4.2 Risk of bias assessment

Full results of the risk of bias assessment can be found in Table 7.3. Studies scored highly on items related to *reporting*, with the exception of reporting adverse events, and *internal validity*. Seven studies (Johnson *et al.*, 2009; Hallsworth *et al.*, 2011; Hickman *et al.*, 2013; Cassidy *et al.*, 2016; Cuthbertson *et al.*, 2016; Langleite *et al.*, 2016; Zhang *et al.*, 2016) blinded the investigators performing assessments of IHTG, but allocation concealment was only performed in three out of 10 RCTs (Cassidy *et al.*, 2016; Langleite *et al.*, 2016; Zhang *et al.*, 2016). Conversely, studies scored poorly in relation to *external validity*, primarily due to an inability to determine whether recruited participants were representative of the entire population or of those who were approached to participate. One study (Keating *et al.*, 2015) performed a power calculation on IHTG to detect change between groups indicating that 116 participants were required per group. However, due to limited data to inform this power calculation and the difficulties in performing a study of that design in such large numbers they did not recruit to this extent. Given that 1) the purpose of this meta-analysis was not to determine differences between groups, 2) they had attempted to perform an appropriate calculation and 3) the numbers they did recruit were similar to those of other suitably powered studies, a score of two was awarded for item 27 (statistical power). Funnel plots suggested minimal evidence of publication bias (Figure 7.2a-c), although the plot of all intervention groups investigating IHTG (Figure 7.2a) suggested a small bias towards studies reporting small effects. This would, if anything, result in an attenuated pooled effect.

Seven studies recorded energy intake using self-reported food diaries (Johnson *et al.*, 2009; Haus *et al.*, 2013; Oh *et al.*, 2014; Pugh *et al.*, 2014; Keating *et al.*, 2015; Cuthbertson *et al.*, 2016) or a validated food frequency questionnaire (Langleite *et al.*, 2016). All of these reported no change in energy intake from pre- to post-intervention, although one (Keating *et al.*, 2015) reported a small increase in carbohydrate intake in participants completing a high-intensity, low-volume exercise programme. One study (Lee *et al.*, 2013) prescribed a weight maintenance diet to participants. A further 10 studies instructed participants to maintain their dietary habits throughout the duration of study involvement without formally monitoring diet (Shojaee-Moradie *et al.*, 2007; van der Heijden, Wang, Chu, Sauer, *et al.*, 2010; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010; Hickman *et al.*, 2013; Malin, Mulya, *et al.*, 2013; Hallsworth *et al.*, 2015; Cassidy *et al.*, 2016; Zhang *et al.*, 2016; Houghton *et al.*, 2017; Sargeant, Bawden, *et al.*, 2018), whilst three manuscripts make no reference to the control of dietary intake (Meex *et al.*, 2010; Hallsworth *et al.*, 2011; Sullivan *et al.*, 2012).

Table 7.3 Risk of bias assessment for all studies (legend overleaf)

First Author (Publication Year)	*Item Category / Number*																								
	Reporting										***External Validity***			***Internal Validity: Bias****						***Internal Validity: Confounding***						***Power***
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***15***	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***23***	***24***	***25***	***26***	***27***
Cassidy (2016)	1	1	1	1	2	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Cuthbertson (2016)	1	1	1	1	2	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	0	1	0	0	1	2
Hallsworth (2011)	1	1	1	1	2	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	0	1	0	0	1	2
Hallsworth (2015)	1	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	1	0	0	1	2
Haus (2013)	1	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	0	0	0	0	1	0
Hickman (2013)	1	1	1	1	2	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	1	2
Houghton (2017)	1	1	1	1	2	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	0	1	0	0	1	2
Johnson (2009)	1	1	1	1	2	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	0	1	0	1	1	0
Keating (2015)	1	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	0	0	0	1	2
Langleite (2016)	1	1	1	1	2	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	0
Lee (2013)	0	1	1	1	2	1	1	0	1	1	0	0	1	0	0	1	1	1	1	1	1	1	0	1	1	0
Malin (2013)	1	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	0	0	0	1	0
Meex (2010)	0	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	0	0	1	1	0
Oh (2014)	1	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	0	1	1	1	0	0	0	1	0
Pugh (2014)	1	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	0	1	0	0	1	1
Sargeant (2018)	1	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	0	0	0	1	0
Shojaee-Moradie (2007)	0	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	1	0	0	1	0
Sullivan (2012)	1	1	1	1	2	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	0	1	0	0	1	2
van der Heijden (2009 & 2010a)[‡]	1	1	1	1	2	1	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	0	0	0	1	0
van der Heijden (2010b)	1	1	1	1	2	1	1	0	1	0	0	0	1	0	0	1	1	1	1	1	0	0	0	1	1	2
Zhang (2016)	1	1	1	1	2	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	2

Full marking criteria can be found in the appendix of the original article by Downs and Black (Downs and Black, 1998). * Item 14 of the Downs and Black scale (concerning participant blinding) was not scored in this meta-analysis as blinding participants to group allocation in exercise studies is very difficult and uncommon. Item 27 (concerning power) was amended as outlined above. A higher number represents a more positive score. ‡ Two manuscripts reporting separate outcomes of the same study.

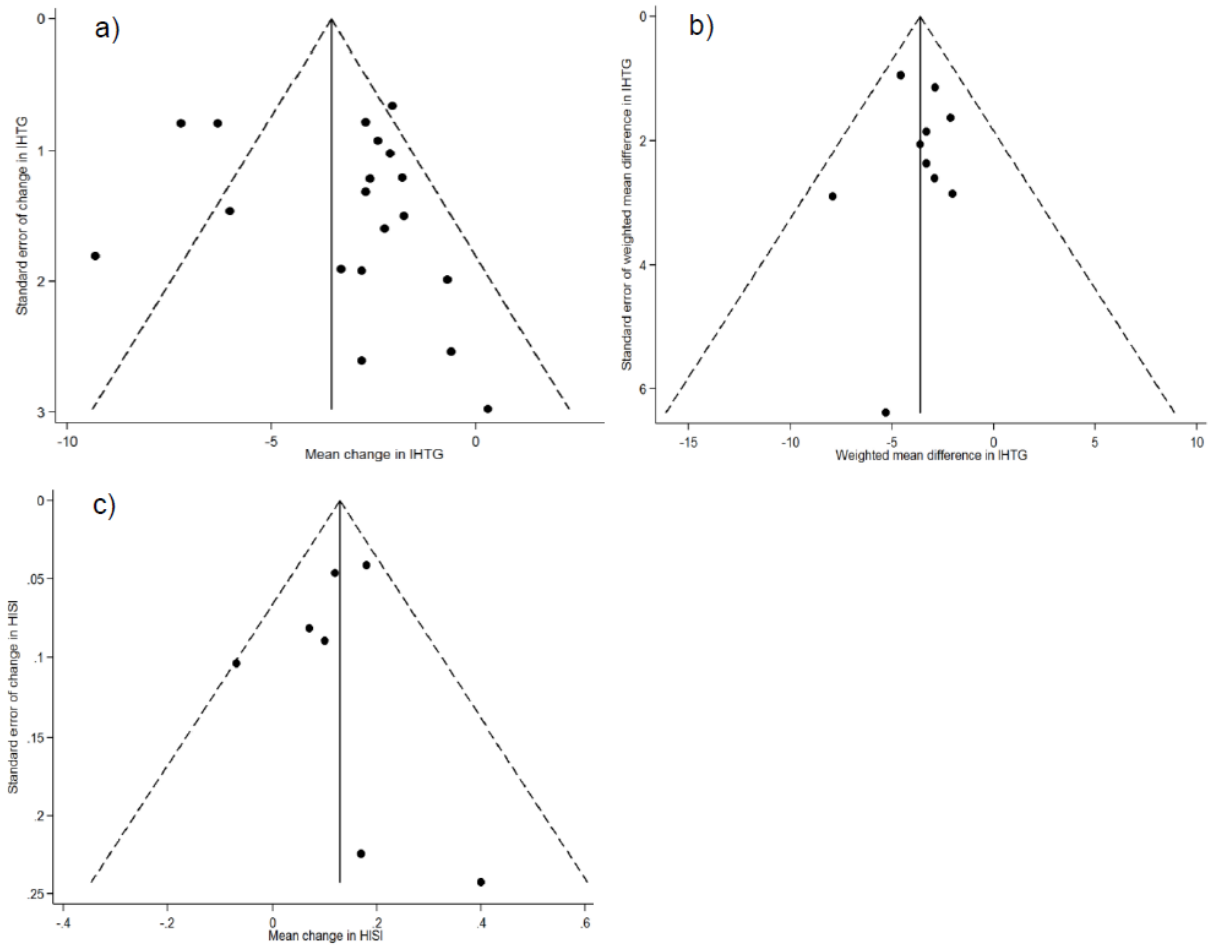


Figure 7.2 Funnel plots for assessment of publication bias in studies included in the following analyses: a) within-group change in IHTG, b) difference between groups in change in IHTG, and c) within-group change in HISI

7.4.3 The effects of exercise training on IHTG

Eighteen studies reported the effects of exercise training on IHTG (Johnson *et al.*, 2009; van der Heijden, Wang, Chu, Sauer, *et al.*, 2010; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010; Hallsworth *et al.*, 2011, 2015; Sullivan *et al.*, 2012; Malin, Mulya, *et al.*, 2013; Haus *et al.*, 2013; Hickman *et al.*, 2013; Oh *et al.*, 2014; Pugh *et al.*, 2014; Keating *et al.*, 2015; Cassidy *et al.*, 2016; Cuthbertson *et al.*, 2016; Langleite *et al.*, 2016; Houghton *et al.*, 2017; Sargeant, Bawden, *et al.*, 2018) (Table 7.2). Only one of these used paired liver biopsy (Hickman *et al.*, 2013), reporting no significant effect of 6-months resistance exercise on the percentage of hepatocytes affected by steatosis in individuals with NAFLD. The remaining 17 studies used ^{1}H-MRS to measure IHTG so, to reduce heterogeneity, only these studies were included in subsequent meta-analyses. These studies contained 19 exercise groups and a combined total of 373 participants (male: 151 [40.5%]; female: 182 [48.8%]; data not reported: 40 [10.7%]). Participants had a weighted mean age of 50 [range 15.5 to 60] years and were overweight or obese (body mass index: 30.6 [27.8 to 37.1] kg·m^{-2}; body fat percentage: 35.6 [28.7 to 43.7] %; waist circumference: 101.2 [95.2 to 111.9] cm). Participants were reported as sedentary and/or inactive and had low aerobic capacity (peak oxygen uptake: 25.2 [21.8 to 38.7] ml·kg^{-1}·min^{-1}). Two studies actively recruited individuals with T2DM (Cassidy *et al.*, 2016) or dysregulated glucose metabolism (Langleite *et al.*, 2016), whilst the mean baseline characteristics of seven other studies (Johnson *et al.*, 2009; Hallsworth *et al.*, 2011, 2015; Haus *et al.*, 2013; Malin, Mulya, *et al.*, 2013; Zhang *et al.*, 2016; Houghton *et al.*, 2017) met diagnostic criteria for impaired fasted glucose (weighted mean fasted glucose: 5.61 [4.09 to 6.80] mmol·L^{-1}) (American Diabetes Association, 2018). The weighted mean IHTG at baseline was 15.8 [6.9 to 23.1] %.

Interventions included aerobic (n = 11), HIIT (n = 3), resistance (n = 2), combined aerobic/HIIT-plus-resistance (n = 2) and acceleration/vibration (n = 1) exercise training, ranging from seven days to 24 weeks (mode: 12 weeks). Session frequency ranged from two to seven times per week (mode: three times per week) for 30 to 60 minutes. Six aerobic interventions used moderate-intensity exercise whilst the remaining five, along with all of the HIIT interventions, were categorised as high-intensity.

Ten of the included studies were RCTs, containing a combined 283 and 169 participants in exercise and control groups, respectively (Table 7.2). Pooled participant and intervention characteristics of RCTs only can be found in Tables 7.4 and 7.5. There were no significant

differences between the pooled characteristics of RCTs and those of all eligible studies ($P \geq 0.13$). Participants in RCT control groups were instructed to maintain standard care (n = 5) or habitual lifestyle activities (n = 1), prescribed a low-intensity stretching programme (n = 2) or attended sessions providing education on the health benefits of exercise (n = 2).

Table 7.4 Pooled participant characteristics of RCTs included in meta-analysis of the effects of exercise training on IHTG (n = 10 studies)

	Exercise Groups	Control Groups
Total participant number	283	169
Male/Female/NR	111/149/23	77/77/15
Age (years)	52 (45 to 61)	52 (39 to 62)
Body weight (kg)	82.7 (71.4 to 103.0)	84.0 (72.1 to 113.7)
BMI ($kg \cdot m^{-2}$)	30.2 (28.0 to 37.1)	30.2 (28.0 to 40.0)
Body fat (%)	34.4 (30.4 to 38.9)	34.8 (31.0 to 42.5)
Waist circumference (cm)	99.9 (95.5 to 110.0)	99.2 (93.7 to 109.0)
Fasted glucose ($mmol \cdot L^{-1}$)	5.63 (4.30 to 6.80)	5.67 (4.00 to 7.00)
$\dot{V}O_2$ peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	23.6 (21.8 to 26.4)	22.3 (18.5 to 27.0)
IHTG (%)	16.2 (6.9 to 21.3)	14.5 (7.1 to 21.4)

Data presented as weighted mean with range.

Table 7.5 Pooled intervention characteristics of RCTs included in meta-analysis of the effects of exercise training on IHTG (n = 10 studies)

	Mode	Range
Intervention duration (weeks)	12	4 to 24
Session frequency (times per week)	3	3 to 5
Session duration (min)	-	30 to 53

Fourteen of the 17 studies reported a statistically significant benefit of exercise on IHTG either within-group (van der Heijden, Wang, Chu, Sauer, *et al.*, 2010; Oh *et al.*, 2014; Pugh *et al.*, 2014; Langleite *et al.*, 2016; Sargeant, Bawden, *et al.*, 2018), between-group (Johnson *et al.*, 2009; Sullivan *et al.*, 2012), or both (Hallsworth *et al.*, 2011, 2015; Keating *et al.*, 2015; Cassidy *et al.*, 2016; Cuthbertson *et al.*, 2016; Zhang *et al.*, 2016; Houghton *et al.*, 2017). In the two studies with multiple exercise groups, exercise elicited a significant reduction in both groups (Keating *et al.*, 2015; Zhang *et al.*, 2016). One RCT reported a significant reduction from baseline, but this was not significant when compared to the change in the control group (Pugh *et al.*, 2014). Of the three studies that reported no benefit of exercise, two were short interventions (performing exercise on seven consecutive days) (Haus *et al.*, 2013; Malin, Mulya, *et al.*, 2013), whilst the other was a resistance exercise programme in obese adolescents (van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010).

When data from pre- to post-exercise in all interventions were pooled, a statistically significant benefit of exercise training was found (Figure 7.3), but high heterogeneity was also apparent. In a sensitivity analysis of RCTs only, the significant benefit of exercise was strengthened (mean difference in change between groups [95% CI]: -3.61 [-4.68 to -2.54] %; Figure 7.4), and results were highly homogeneous ($I^2 < 0.1\%$, $P = 0.84$). Therefore, to allow the inclusion of maximum data, subgroup analyses and meta-regressions were performed using the within-group change in all interventions.

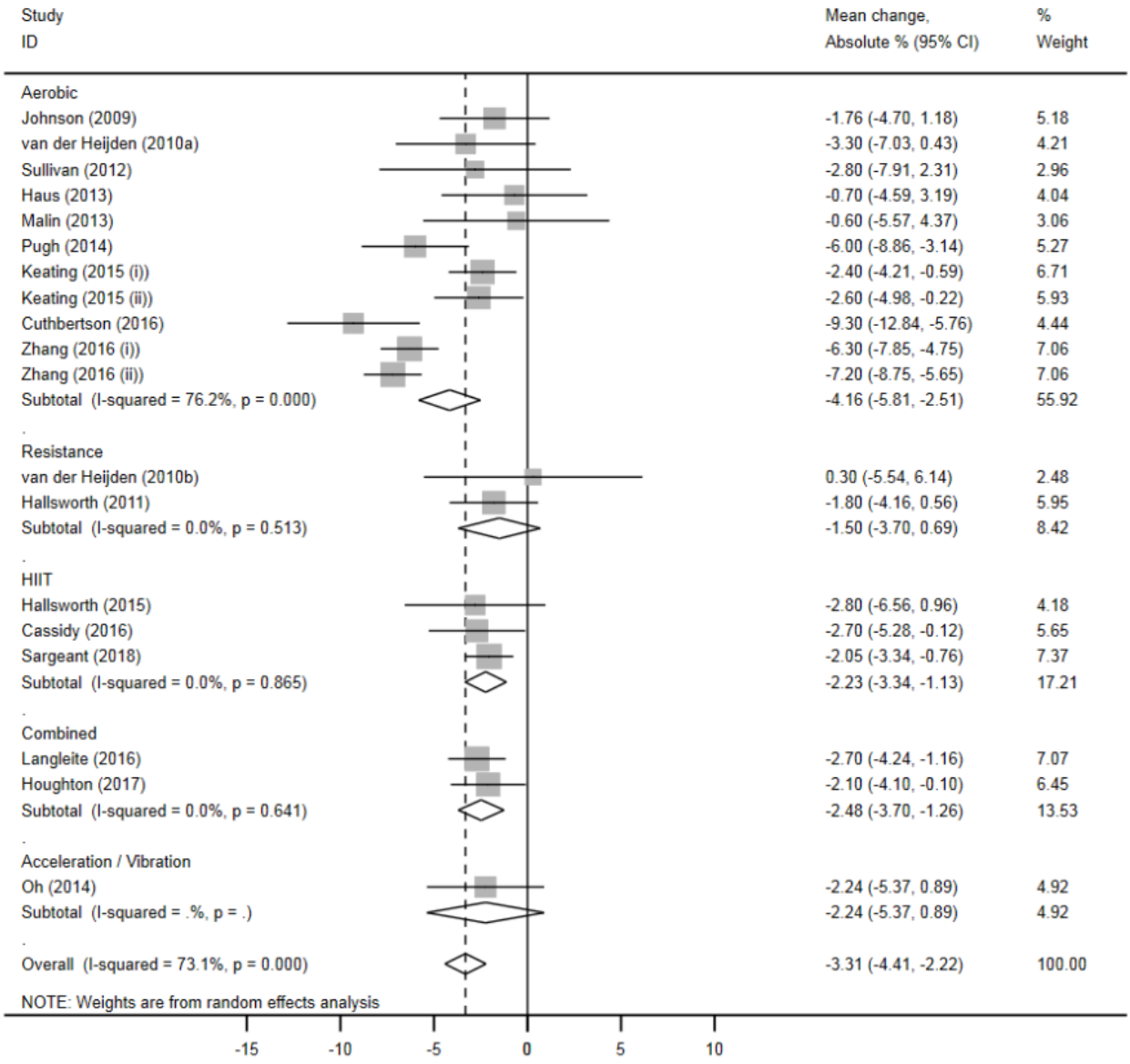


Figure 7.3 Meta-analysis of the pooled effect of exercise training on IHTG from pre- to post-training in all exercise groups of all eligible studies. Studies are grouped by exercise mode.

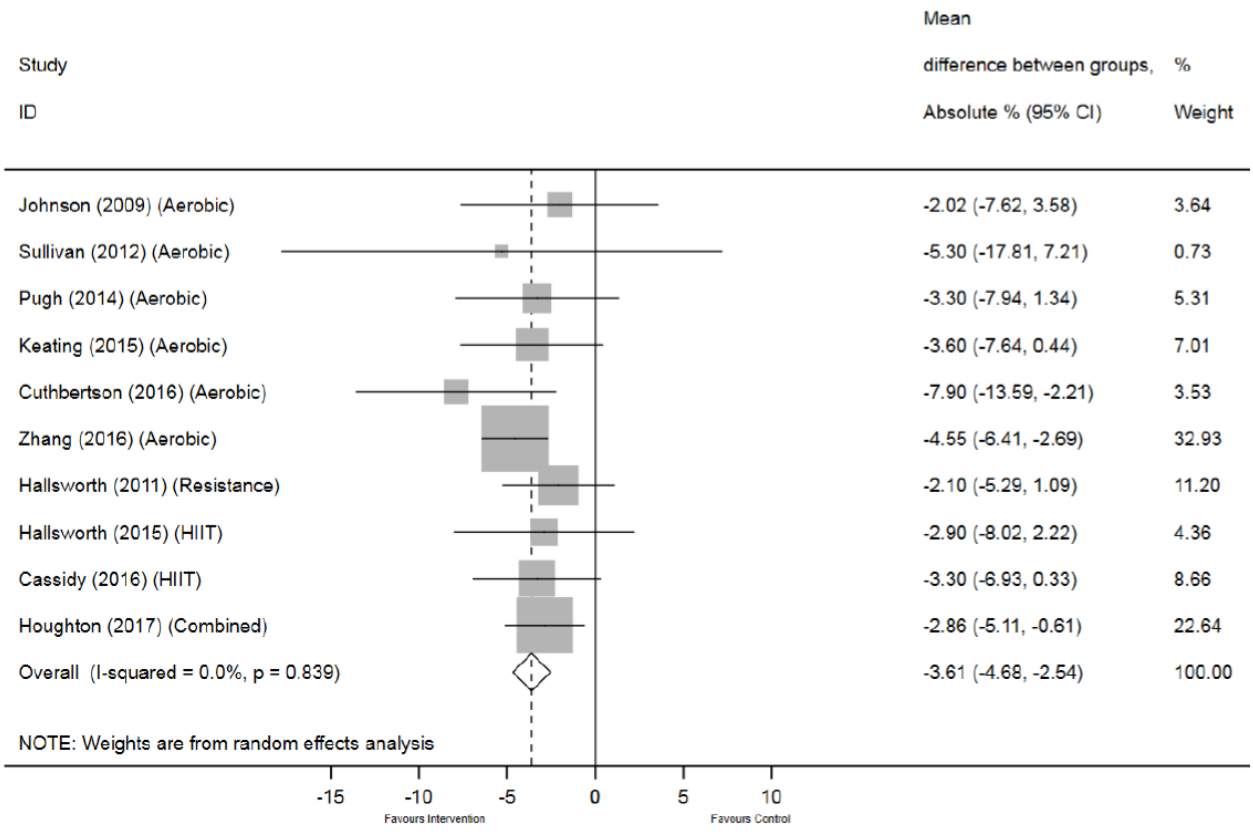


Figure 7.4 Meta-analysis of the pooled effect of exercise training on IHTG using eligible RCTs only

Exercise training significantly reduced IHTG in the absence of weight loss. However, when significant weight loss occurred, the pooled effect was substantially greater (Figure 7.5). Furthermore, meta-regression displayed a significant positive relationship between the change in body weight (relative to baseline) and the absolute change in IHTG (β = 0.99 [95% CI: 0.62 to 1.36], P < 0.001; Figure 7.6). A significant relationship was also apparent between intervention duration and change in IHTG (β = -0.27 [95% CI: -0.35 to -0.19], P < 0.001; Figure 7.7), suggesting that as the duration of intervention increases, so does the magnitude of reduction in IHTG. The duration of interventions that elicited significant weight loss versus those that did not were similar (median [range]: 12 [8 to 24] vs. 12 [1 to 16] weeks), but bivariate Pearson's correlation analysis showed a significant positive relationship between the duration of intervention and magnitude of weight loss elicited (r^2 = 36%, P < 0.01).

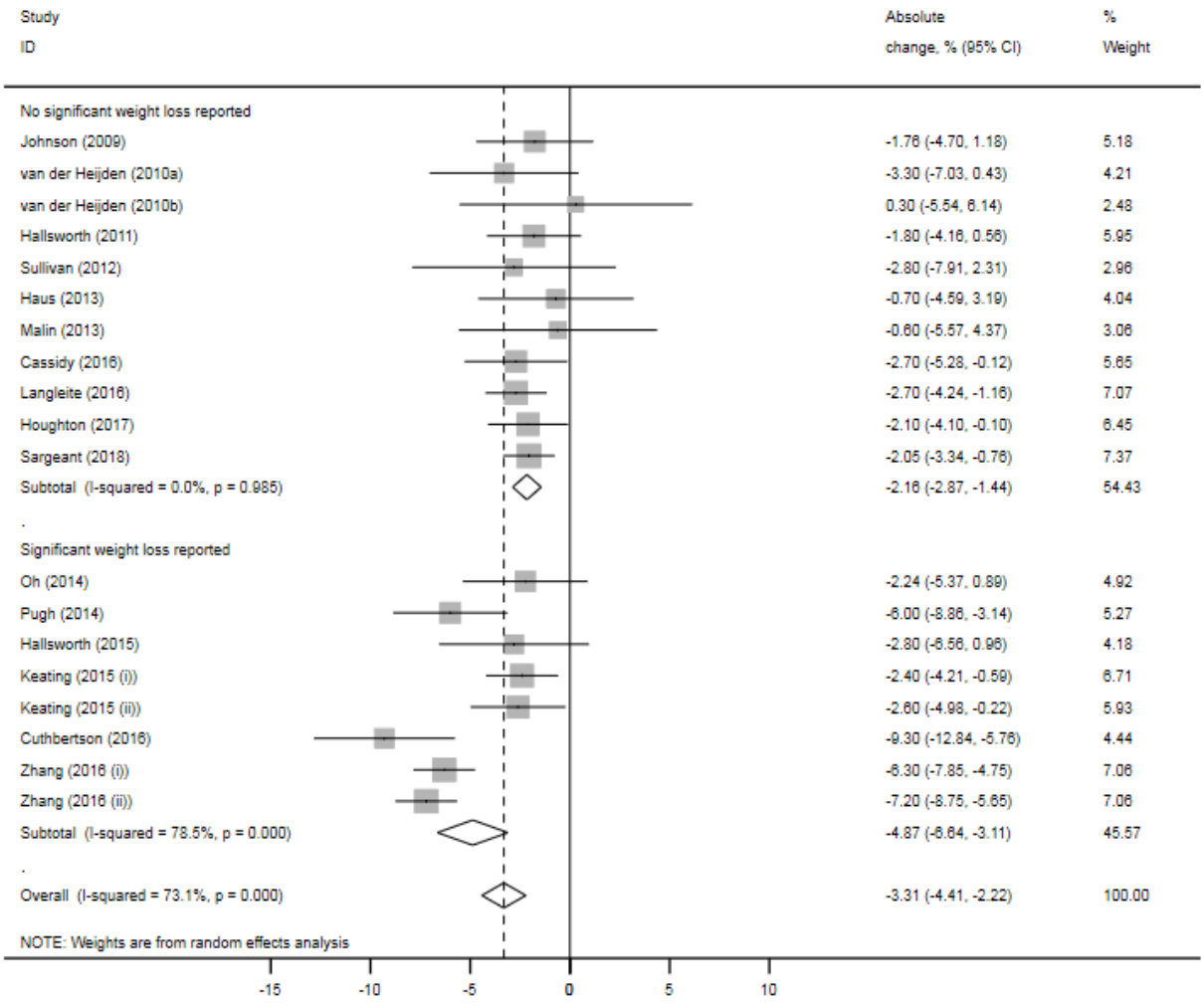


Figure 7.5 Meta-analysis of the pooled effect of exercise training on IHTG from pre- to post-training in all exercise groups of all eligible studies when studies are grouped into those that elicited weight loss versus those that did not.

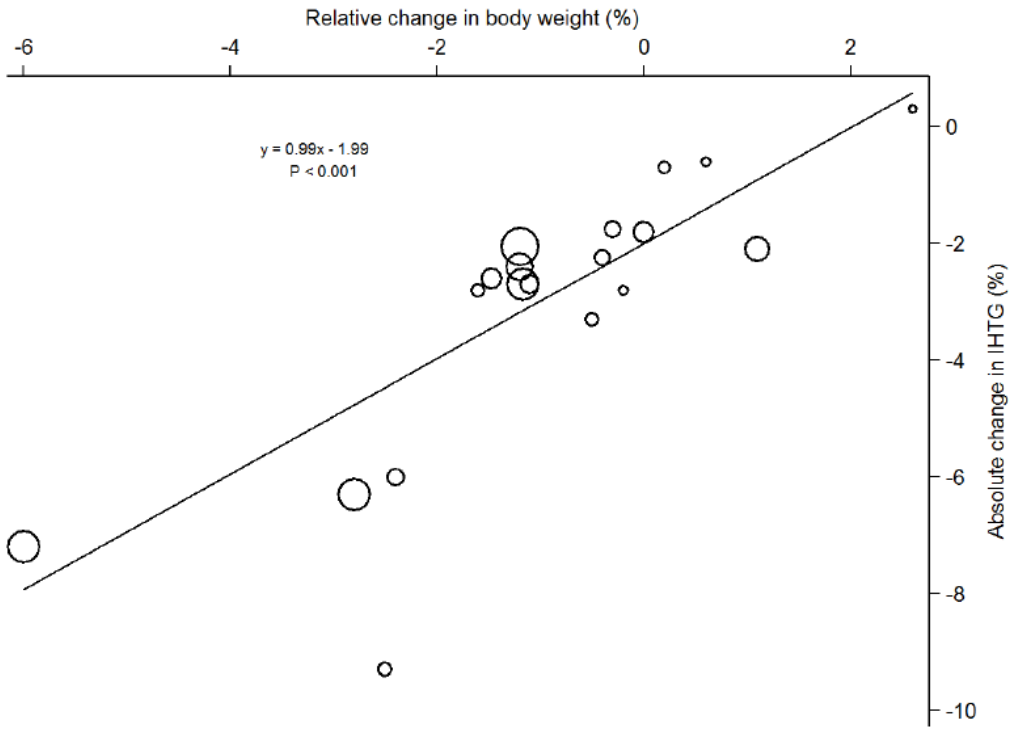


Figure 7.6 Meta-regression between the magnitude of weight loss, relative to baseline, elicited by exercise intervention and the absolute change in IHTG. Each circle represents a study, with larger circles indicating greater weight within the meta-regression analysis.

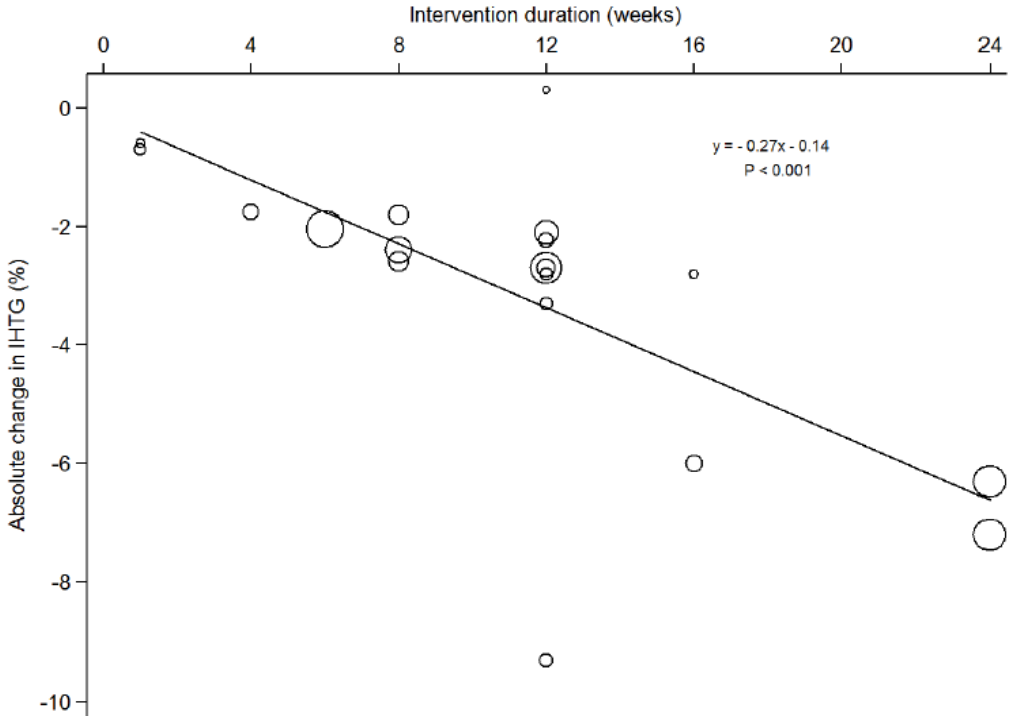


Figure 7.7 Meta-regression between duration of exercise intervention in weeks and the absolute change in IHTG from baseline elicited in all eligible studies. Each circle represents a study, with larger circles indicating greater weight within the meta-regression analysis.

Figure 7.3 displays the mean change from baseline in IHTG for all interventions categorised by exercise type. The pooled effect on IHTG for aerobic exercise interventions was greater than that for each other mode of exercise, as well as the pooled mean for all interventions. However, the high prevalence of aerobic interventions in comparison to other types of intervention should be noted. When interventions were grouped according to exercise intensity, the pooled effect for moderate-intensity interventions was greater than that of the high-intensity exercise programmes (-4.82 [-7.00 to -2.65] %, I^2 = 75.5%, P = 0.001 vs. -2.89 [-4.25 to -1.53] %, I^2 = 73.2%, P < 0.001). The intervention duration of the moderate and high-intensity interventions were similar (median [range]: 14 [4 to 24] vs. 12 [1 to 24] weeks).

7.4.4 Overview of studies investigating the effects of exercise on hepatic insulin sensitivity

Eight studies, containing a total of 10 exercise groups, reported the effects of exercise training on hepatic insulin sensitivity (Shojaee-Moradie *et al.*, 2007; van der Heijden *et al.*, 2009; Meex *et al.*, 2010; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010; Hickman *et al.*, 2013; Lee *et al.*, 2013; Cuthbertson *et al.*, 2016; Sargeant, Bawden, *et al.*, 2018) (Table 7.2). Four studies reported HISI (van der Heijden, Wang, Chu, Sauer, *et al.*, 2010; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010; Lee *et al.*, 2013; Sargeant, Bawden, *et al.*, 2018), although three presented EGP (and thus HISI) in different units to those outlined above. Two studies reported HIRI (Hickman *et al.*, 2013; Cuthbertson *et al.*, 2016). Four studies were able to provide raw data for the re-calculation of HISI (van der Heijden, Wang, Chu, Sauer, *et al.*, 2010; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010; Hickman *et al.*, 2013; Cuthbertson *et al.*, 2016), along with two that reported EGP and FPI separately (Shojaee-Moradie *et al.*, 2007; Meex *et al.*, 2010). As such, seven studies (eight exercise groups) were included in meta-analysis of changes in HISI with exercise training. Only two of these (Shojaee-Moradie *et al.*, 2007; Cuthbertson *et al.*, 2016) were RCTs with a non-intervention/standard care control group (containing a combined 22 and 14 participants in exercise and control groups, respectively) so only within-group analysis was conducted. Three studies also reported $\%EGP_{supp}$ with low-dose insulin infusion (Shojaee-Moradie *et al.*, 2007; Cuthbertson *et al.*, 2016; Sargeant, Bawden, *et al.*, 2018) but, due to this limited number, meta-analysis was not performed. Seven further studies were found that utilised a study design allowing the calculation of at least one of HISI or $\%EGP_{supp}$, but neither were reported (DeFronzo, Sherwin and Kraemer, 1987; Segal *et al.*, 1991; Hughes *et al.*, 1993; Coker *et al.*, 2006, 2009; DiPietro *et al.*, 2006; Kirk, Sullivan and Klein, 2010). Whilst the authors of five of these studies kindly replied to requests for raw data, none were able to provide it.

7.4.5 The effects of exercise training on basal hepatic insulin sensitivity

The study that was excluded from meta-analysis of changes in HISI reported a tendency for a reduction in HIRI in obese adolescent girls following 12 weeks of resistance exercise training, but not after 12 weeks of moderate- to high-intensity aerobic exercise (Lee *et al.*, 2013). The remaining eight exercise groups were pooled and had a combined total of 105 participants (male: 84 (80%); female: 20 (19%); data not available: 1 (1%)). Participants had a weighted mean age of 43 [range 15.5 to 59] years and, as per study selection criteria, were overweight or obese (body mass index: 31.3 [27.6 to 35.3] $kg \cdot m^{-2}$; body fat percentage: 34.0 [25.5 to 40.8] %). Participants were reported as sedentary and/or inactive and had low aerobic capacity (maximal or peak oxygen uptake: 24.7 [21.6 to 32.0] $ml \cdot kg^{-1} \cdot min^{-1}$). Five studies (five exercise groups) were conducted in individuals with diagnosed NAFLD or in those with baseline characteristics that met diagnostic criteria as outlined above (van der Heijden *et al.*, 2009; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010; Hickman *et al.*, 2013; Cuthbertson *et al.*, 2016; Sargeant, Bawden, *et al.*, 2018). One study included separate groups of patients with impaired fasted glucose and T2DM (Meex *et al.*, 2010) but, according to mean baseline characteristics, all other studies recruited individuals with normal fasted glycaemia (weighted mean fasted glucose for all studies was 5.36 [4.70 to 9.00] $mmol \cdot L^{-1}$) (American Diabetes Association, 2018). The weighted mean HISI at baseline was 0.99 [0.58 to 2.09] $mg \cdot m^{-2} \cdot min^{-1}$ per $\mu U \cdot mL^{-1}$.

Studies included aerobic ($n = 3$), HIIT ($n = 1$), resistance ($n = 2$) and combined ($n = 2$) exercise interventions for six ($n = 2$), 12 ($n = 5$) or 24 ($n = 1$) weeks. Participants exercised on two to four days per week with sessions ranging from 20 to 60 minutes. One aerobic intervention, and the aerobic component of the combined exercise programmes, utilised moderate-intensity exercise, whilst the remaining aerobic interventions and the HIIT intervention were categorised as high-intensity.

Two of the eight exercise groups displayed a statistically significant improvement in HISI with exercise, both of which were in adolescents (van der Heijden *et al.*, 2009; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010). One study reported a tendency for reduced HIRI after exercise training, but this effect was statistically significant when HISI was re-calculated as above. Neither RCT reported a significant difference between exercise and control groups (Shojaee-Moradie *et al.*, 2007; Cuthbertson *et al.*, 2016). However, when data were pooled, a statistically significant benefit of exercise was found (Figure 7.8). Based on the weighted mean

HISI at baseline, this pooled effect represents a relative improvement of approximately 13%. Two studies in obese adolescents reported a reduction in basal EGP after aerobic or resistance exercise training (van der Heijden *et al.*, 2009; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010), but EGP was unaffected in all other studies. In contrast, FPI was significantly reduced in five of the exercise groups (van der Heijden *et al.*, 2009; Meex *et al.*, 2010; Cuthbertson *et al.*, 2016; Sargeant, Bawden, *et al.*, 2018), whilst the mean reduction in another (Hickman *et al.*, 2013) approached statistical significance. One study (Cuthbertson *et al.*, 2016) reported a statistically significant reduction in body weight from pre- to post-exercise training (2.5%) but this was not associated with an improvement in HISI. Another study (Hickman *et al.*, 2013) reported a mean reduction in body weight of similar magnitude (2.6%) which, although not statistically significant, was associated with a significant improvement in HISI.

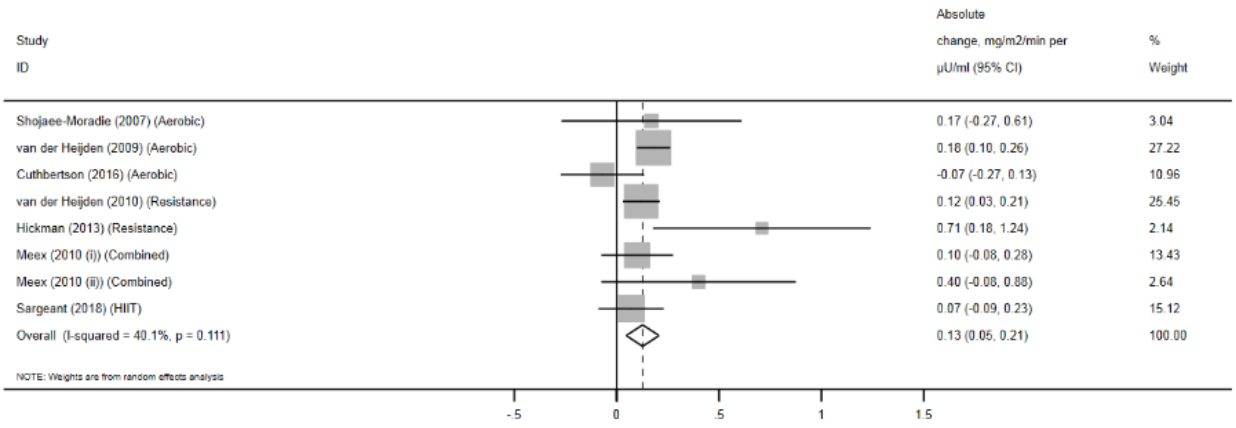


Figure 7.8 Meta-analysis of the pooled effect of exercise training on HISI from pre- to post-training in all exercise groups of all eligible studies

7.4.6 The effects of exercise training on insulin-stimulated hepatic insulin sensitivity

Participant and intervention characteristics for the three studies examining the effects of exercise on $\%EGP_{supp}$ can be found in supplementary materials (Appendix XI). The two RCTs that reported HISI also reported $\%EGP_{supp}$. One RCT reported a significant improvement in $\%EGP_{supp}$, compared to the control group, after six weeks of aerobic exercise training, despite no change in total body weight or IHTG (Shojaee-Moradie *et al.*, 2007). In contrast, $\%EGP_{supp}$ was reportedly unaffected by either twelve weeks of aerobic training (Cuthbertson *et al.*, 2016) or six weeks of HIIT (Sargeant, Bawden, *et al.*, 2018).

7.5 Discussion

The prominent findings of this review are that structured exercise training, independent of dietary intervention, reduces IHTG in individuals with NAFLD. Importantly, whilst this effect is apparent without weight loss, the magnitude of reduction increases in direct proportion to the amount of weight loss induced by the intervention. Furthermore, our analyses indicate that exercise training for six to 24 weeks may also improve basal hepatic insulin sensitivity by approximately 13%.

Previous reviews report that exercise training significantly reduces IHTG with a moderate to large pooled effect size (Keating *et al.*, 2012; Orci *et al.*, 2016; Katsagoni *et al.*, 2017). The restriction of the current meta-analysis to a single technique (^{1}H-MRS) allowed us to report this pooled effect as the absolute mean difference and our findings suggest that exercise training for between one and 24 weeks (mode 12 weeks) elicits an absolute reduction in IHTG of approximately 2.2 to 4.7% (mean ~3.5%); re-enforcing the meaningful therapeutic role of exercise for individuals with NAFLD. An important finding to emerge from this meta-analysis is the impact of weight loss as a mediator of the reduction in IHTG associated with exercise training interventions. This outcome is consistent with previous reviews (Brouwers *et al.*, 2016; Golabi *et al.*, 2016), which have highlighted the more potent impact of weight loss *per se* (primarily through dietary energy restriction) than the independent effect of exercise in the absence of body weight reduction. Specifically, our data show that whilst IHTG is significantly reduced in the absence of exercise-induced weight loss, the magnitude of effect is more than two-fold greater when weight loss occurs (-2.16 vs. -4.87%). Meta-regression suggests that each 1% relative reduction in body weight is associated with approximately 1% absolute reduction in IHTG. Interestingly, an almost identical relationship can be seen in studies that have examined IHTG responses to short-, medium- and long-term dietary energy restriction interventions (Browning *et al.*, 2011; Wong *et al.*, 2013; Patel *et al.*, 2015). These data therefore illustrate that relative changes in IHTG are several-fold greater than associated alterations in body weight regardless of physiological stimulus. This overriding influence of weight loss may also explain why previous reviews have not found additive benefits of exercise when combined with dietary intervention (Keating *et al.*, 2012; Smart *et al.*, 2016). In these scenarios, large energy deficits created by dietary modification may dilute the much smaller contribution of exercise (Ross, Freeman and Janssen, 2000). Consequently, whilst exercise alone may be effective at reducing IHTG, the greatest therapeutic benefits will be realised when exercise interventions contribute to weight loss in combination with dietary energy restriction.

These findings have direct implications for exercise prescription in NAFLD. Firstly, given that weight loss appears to be the dominant factor mediating changes in IHTG (as demonstrated in our meta-regression analyses), and the capacity of dietary energy restriction to induce large amounts of weight loss is greater than that of exercise training (Browning *et al.*, 2011; Wong *et al.*, 2013; Patel *et al.*, 2015), structured exercise training should be seen as a valuable addition, rather than an alternative, to dietary energy restriction in the majority of individuals. That said, whilst the magnitude of effects may be comparatively smaller, there is evidence to suggest weight-independent effects of exercise training on IHTG, including beneficial changes in skeletal muscle and hepatic substrate metabolism (Brouwers *et al*., 2016). Therefore, the benefits of exercise on IHTG should not be completely dismissed and structured exercise training may be an effective alternative for individuals where prolonged energy restriction has proved ineffective. No studies to date have directly compared the impact of the diet- versus exercise-induced weight loss, matched for magnitude, on IHTG. Notably, the wider benefits of exercise training on metabolic health beyond IHTG should also not be forgotten (Garber *et al*., 2011).

When recommending exercise training for individuals with NAFLD, the greatest impact of exercise training on IHTG is likely to occur with exercise that is associated with the greatest weight loss (Smart *et al.*, 2016). Evidence suggests that aerobic exercise interventions typically evoke a higher amount of weight loss than anaerobic exercise modalities (Yumuk *et al.*, 2015). The superiority of aerobic exercise interventions for reducing IHTG in individuals with NAFLD is supported by the present results and those of a previous meta-analysis (Orci *et al.*, 2016). Consequently, although resistance exercise training and HIIT promote a reduction in IHTG in patients with NAFLD, greater benefits will likely be achieved through continuous aerobic exercise protocols such as running, swimming and cycling.

Exercise volume may also be an important variable which helps to explain why our analyses documented a larger reduction in IHTG with moderate- compared to high-intensity exercise interventions. Specifically, compared with shorter intense bouts of exercise, continuous moderate-intensity protocols commonly exhibit a greater total exercise volume and, consequently, energy expenditure. This higher level of energy expenditure may elicit a greater impact on metabolism, energy balance and IHTG. It is, however, important to consider that exercise-related energy expenditure may not necessarily translate into greater total daily energy expenditure (Pontzer *et al.*, 2016; Melanson, 2017). Furthermore, given the inability to accurately calculate exercise-related energy expenditure in our review, further research is

needed to investigate the precise relationship between exercise-related energy expenditure and IHTG.

Our analyses also demonstrate a significant relationship between the duration of exercise interventions and the change in IHTG, and a positive relationship between the duration of exercise training and the magnitude of weight loss. Each week of exercise training is associated with a reduction in IHTG of approximately 0.27%. Collectively, our findings highlight the importance of developing sustainable exercise interventions, which, in combination with dietary strategies, may elicit the greatest benefits on IHTG in individuals with NAFLD.

The second part of this review examined the influence of exercise training on hepatic insulin sensitivity. Hepatic insulin resistance, which is strongly correlated with elevated IHTG (Korenblat *et al.*, 2008; Shulman, 2014; Bril, Barb, *et al.*, 2017), contributes to impaired glycaemic control in the pathogenesis of T2DM (Taylor, 2008; Rizza, 2010; Petersen, Vatner and Shulman, 2017). We assessed the impact of exercise interventions on HISI and $\%EGP_{supp}$, which are measures of hepatic insulin sensitivity in the basal and insulin-stimulated states, respectively. The paucity of studies examining these effects meant that our analyses were conducted using any study that recruited individuals with overweight or obesity, who are thus at increased risk of NAFLD, in addition to studies exclusively in patients with NAFLD.

In total, we identified eight studies (two of which had two exercise groups) which assessed the impact of exercise on basal hepatic insulin sensitivity (Shojaee-Moradie *et al.*, 2007; van der Heijden *et al.*, 2009; Meex *et al.*, 2010; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010; Hickman *et al.*, 2013; Lee *et al.*, 2013; Cuthbertson *et al.*, 2016; Sargeant, Bawden, *et al.*, 2018). Seven studies had sufficient data for inclusion in our meta-analysis but only two of which were RCTs. Therefore, our analysis was restricted to within-group changes in eight exercise groups. Interestingly, despite only three exercise groups displaying significant improvements in HISI from pre- to post- intervention (van der Heijden *et al.*, 2009; van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010; Hickman *et al.*, 2013), our pooled analysis identified a statistically significant increase of approximately 13%. In adults, basal rates of EGP were unaffected by exercise, but this improvement in basal hepatic insulin sensitivity may be reflected by a reduction of FPI. The clinical relevance of this magnitude of improvement in basal hepatic insulin sensitivity for individuals that are overweight and obese is not immediately clear. However, this novel finding suggests that exercise training favourably modifies this important parameter in individuals at risk of NAFLD and T2DM.

Three studies reported the effects of exercise on $\%EGP_{supp}$ (Shojaee-Moradie *et al.*, 2007; Cuthbertson *et al.*, 2016; Sargeant, Bawden, *et al.*, 2018), two of which were RCTs employing aerobic exercise interventions of six and 12 weeks (Shojaee-Moradie *et al.*, 2007; Cuthbertson *et al.*, 2016). While one study reported significant improvement in $\%EGP_{supp}$, no change was reported in the other. Therefore, we cannot draw firm conclusions on the impact of exercise training on insulin-stimulated hepatic insulin sensitivity at this time.

It is notable that we identified a further seven studies whose methods permitted the calculation of HISI or $\%EGP_{supp}$ but data were not presented or available. Furthermore, when hepatic insulin sensitivity was reported, the precise details of the experimental methods employed, units used for presentation of outcomes and calculation of indices of hepatic insulin sensitivity, varied between studies. This review, therefore, highlights the need for greater methodological and reporting consistency when assessing hepatic insulin resistance (e.g. standardisation of low-dose insulin infusion when undertaking dual-stepped hyperinsulinaemic-euglycaemic clamps and consistent use of units).

Our meta-analysis of the effects of exercise training on IHTG in individuals with NAFLD, and the mediating influence of weight loss, is the most precise quantitative synthesis to date. Furthermore, this is the first systematic review and meta-analysis assessing the impact of exercise interventions on hepatic insulin sensitivity. However, a few important considerations are noteworthy. A range of exercise interventions are identified and our analyses of the effects of exercise on IHTG display significant heterogeneity. Whilst we explore potential sources of this heterogeneity, it is assumed that these studies are suitable for data pooling. Secondly, although most included studies attempt to control habitual diet, this is notoriously difficult (Dhurandhar *et al.*, 2015) and the potential for dietary changes to influence study outcomes must, therefore, be recognised. Additionally, our subgroup analyses investigating the influence of weight loss on IHTG were performed with studies categorised according to statistical, rather than clinical, significance. This has the potential to exclude studies from the weight loss group that demonstrate physiologically relevant, but not statistically significant, weight loss. Notably, however, this is unlikely in our analyses because, in the ‘no weight loss’ studies, the largest mean relative reduction in body weight after exercise training was 1.2%. Furthermore, it is assumed that weight loss resulting from exercise training is primarily reflective of a reduction in fat mass but some exercise regimens may promote the synthesis of skeletal muscle; attenuating any reduction in total body weight. It should also be noted that while a reduction in IHTG may be indicative of improved metabolic health, these may be mediated by a reduction

in hepatic lipid intermediates rather than IHTG *per se* (Samuel *et al.*, 2004; Kumashiro *et al.*, 2011; Shulman, 2014). Finally, the limited number of studies investigating hepatic insulin sensitivity means that our findings should be taken with caution. Large RCTs are required to further investigate the effects of exercise training on hepatic insulin sensitivity, particularly in individuals with NAFLD and T2DM.

In conclusion, this systematic review and meta-analysis has shown that exercise training reduces IHTG in individuals with NAFLD and, whilst benefits can be realised in the absence of weight loss, reductions in IHTG are proportionally related to the magnitude of weight loss induced. Furthermore, exercise training may improve basal hepatic insulin sensitivity in individuals that are overweight or obese, which may have beneficial implications for the management of NAFLD and T2DM.

CHAPTER 8

GENERAL DISCUSSION

8.1 Principal findings and chapter overview

Collectively, the body of work presented in this thesis had three primary aims:

1. To examine the effects of acute exercise on several circulating hepatokines, and their associations with anthropometric and circulating clinical biomarkers in individuals of different weight status and glycaemic control.
2. To test the feasibility and efficacy of SIT as a novel intervention for patients with NAFLD, particularly exploring its effects on IHTG and tissue-specific (hepatic and peripheral) insulin sensitivity.
3. To collate the existing literature and summarise the effects of exercise training on IHTG and hepatic insulin sensitivity, exploring the mediating influence of weight loss.

The principal findings of this thesis are that:

1. In volunteers free from chronic metabolic disease and with normal glucose regulation, circulating FGF21 and LECT2 are higher in individuals with overweight or obesity compared to normal weight healthy controls (Chapter 4).
2. In all these individuals, a single bout of CME elicits a transient increase in circulating FGF21 and follistatin (Chapter 4), but whilst the follistatin response is maintained in individuals with dysglycaemia, the response of FGF21 is abolished (Chapter 5).
3. In a controlled, supervised environment, SIT is feasible for individuals with NAFLD and reduces IHTG without significant reduction in body weight. However, hepatic insulin sensitivity is unaffected by SIT and the response of peripheral insulin sensitivity is highly variable (Chapter 6).
4. Whilst exercise training has the potential to reduce IHTG in the absence of weight loss, the greatest benefits are apparent when training is associated with significant reductions in body weight (Chapter 7).
5. However, there is limited evidence examining the effects of exercise training on hepatic insulin sensitivity. The effects in the insulin-stimulated state ($\%EGP_{supp}$) are particularly unclear, but exercise training may improve basal hepatic insulin sensitivity, as assessed using HISI (Chapter 7).

This chapter discusses these findings collectively and, where appropriate, their impact on clinical practice. Some additional analyses are also presented, pooling hepatokine data from Chapters 4, 5 and 6, before potential directions for future investigations are highlighted.

8.2 Intrahepatic triglyceride

The studies in Chapters 6 and 7 of this thesis demonstrate that supervised exercise training alone has the capacity to reduce IHTG in individuals with NAFLD. Importantly, whilst this may occur in the absence of significant weight loss, the magnitude of reduction in IHTG increases in direct proportion with the magnitude of weight loss elicited. It is important to note, however, that the reduction in IHTG with exercise training is modest, even when weight loss occurs.

The systematic review and meta-analysis presented in Chapter 7 collated studies examining the effects of exercise training on IHTG in patients with NAFLD, restricting eligible studies to those using ^{1}H-MRS. This restrictive inclusion criteria was a strength of the study in Chapter 7 because ^{1}H-MRS has much greater precision than alternative methods of assessment, such as US or CT, to assess small changes in IHTG. Furthermore, the use of a single common technique allowed estimates of the pooled effect to be presented in absolute units (% change in IHTG), rather than a standardised effect size. Of the studies identified in Chapter 7, the greatest reduction with exercise training was reported by Cuthbertson and colleagues, where 16 weeks of aerobic exercise training elicited a median absolute reduction in IHTG of 9.3% (Cuthbertson *et al.*, 2016). However, when data from all eligible studies were pooled (total 373 patients with NAFLD), the mean reduction in IHTG over 1 to 24 weeks of exercise training was approximately two-thirds lower (3.3%). This pooled effect was increased in a subgroup analysis of studies reporting associated weight loss, but remained less than 5%. Moreover, the largest (n = 220) and longest study identified in Chapter 7 randomised individuals to one of two 12-month aerobic exercise interventions or a standard-care control (6-month data were used for quantitative data pooling; see Appendix X for justification) (Zhang *et al.*, 2016). This study reported approximately 6.5 to 7% absolute reduction in IHTG at 12 months in both exercise groups (Zhang *et al.*, 2016).

The pooled mean baseline IHTG in Chapter 7 was approximately 16%, which was similar to that reported in the studies by Cuthbertson *et al* (17%) and Zhang *et al* (19%) (Cuthbertson *et al.*, 2016; Zhang *et al.*, 2016). Consequently, the magnitude of reduction in IHTG with exercise training alone (as outlined above) would be insufficient to bring the typical NAFLD patient below diagnostic criteria (5.56%) (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018), at least within 12 months of training. Furthermore, many of the studies identified in Chapter 7 recruited individuals with relatively low disease severity and IHTG may be even higher in individuals

with advanced NAFLD. Accordingly, only one study in Chapter 7 reported a mean IHTG post-training below 5.56% (Cassidy *et al.*, 2016). The baseline IHTG of individuals in this study was comparatively lower (6.9%) than many of the other eligible studies in Chapter 7. Furthermore, in Chapter 6 of this thesis, only two of the nine participants reduced IHTG below 5.56% after six weeks of SIT, and these individuals had pre-intervention IHTG of 5.8% and 8.6%, respectively, which was much lower than the group mean of 14.4%. These data suggest that the independent effect of exercise training alone on IHTG is modest and unlikely to be sufficient to bring the majority of patients with NAFLD below clinical diagnostic criteria.

The modest effect of exercise training on IHTG could be explained, at least in part, by the fact that the weight loss associated with exercise training alone is also low. For example, in the studies by Cuthbertson *et al* and Zhang *et al*, the mean relative reduction in body weight from pre- to post-training was 2.5% and 4%, respectively (Cuthbertson *et al.*, 2016; Zhang *et al.*, 2016). In contrast, dietary energy restriction has the capacity to impart a much greater influence on daily energy balance and is thus associated with much greater reductions in body weight (Ross, Freeman and Janssen, 2000). Accordingly, dietary energy restriction in patients with NAFLD elicits much greater reductions in IHTG than that of exercise training (Petersen *et al.*, 2005; Villareal *et al.*, 2011; Hickman *et al.*, 2013; Washburn *et al.*, 2014). For example, in patients with NASH, approximately 10% weight loss through dietary energy restriction reduces the number of hepatocytes affected by steatosis, determined by histological assessment of liver biopsy tissue, (from 73% to 23%; $P = 0.04$; $n = 5$) and this reduction is much greater than that of a matched group undergoing resistance exercise training without weight loss (71% to 54%, $P = 0.12$; $n = 9$) (Hickman *et al.*, 2013). Furthermore, in patients with T2DM (mean baseline IHTG of 12%), daily energy restriction (total energy intake 1200 kcal per day) elicits 8% weight loss in just seven weeks and this is associated with an approximate 10% absolute reduction in IHTG (Petersen *et al.*, 2005).

Importantly, in Chapter 7, meta-regression analyses suggest that each 1% relative reduction in body weight through exercise training alone is associated with approximately 1% absolute reduction in IHTG. This relationship is similar to that shown previously with dietary restriction-induced weight loss (Petersen *et al.*, 2005; Browning *et al.*, 2011; Wong *et al.*, 2013; Patel *et al.*, 2015). Therefore, whilst the absolute independent effect of exercise training on IHTG may be modest, exercise-associated weight loss may contribute towards substantial reductions in IHTG, when combined with dietary energy restriction. The role of exercise type in modulating reductions in IHTG is discussed in more detail below (*see Section 8.5*).

8.3 Effects of exercise training beyond IHTG

Given the dominance of energy restriction compared to exercise training in its potential to mediate energy balance and reduce IHTG, it is understandable that some individuals may consider the promotion of structured exercise as a lesser priority in patients with NAFLD. However, whilst weight loss remains a core component in the management of NAFLD (and many other chronic metabolic diseases), the importance of exercise should not be readily dismissed. Firstly, dietary approaches may not be tolerated or sustained by all individuals so, whilst less than optimal, exercise training may constitute the primary treatment approach for these patients (alongside pharmacological treatment of other associated co-morbidities). Secondly, exercise has several important benefits beyond its impact on body weight and IHTG, many of which are clinically relevant for patients with NAFLD (Garber *et al.*, 2011; Fiuza-Luces *et al.*, 2013; Keating, George and Johnson, 2015).

Exercise training elicits several beneficial effects on cardiovascular risk factors, such as improved endothelial function, reduced blood pressure and a more favourable circulating lipid profile (Garber *et al.*, 2011; Pugh *et al.*, 2014; Zhang *et al.*, 2016). Whilst some of these benefits may not impact directly on the clinical severity of NAFLD *per se*, CVD remains the leading cause of death in patients with NAFLD, so reduction in cardiovascular risk is an important clinical outcome (Targher, Day and Bonora, 2010; Targher *et al.*, 2016). In Chapter 6, circulating HDL was improved after six weeks of SIT in patients with NAFLD. There were no other statistically significant changes in circulating cardiovascular risk factors, but this may have been a result of the small sample size recruited. Exercise training has also been shown to improve cardiorespiratory fitness and physical function (Garber *et al.*, 2011; Fiuza-Luces *et al.*, 2013; Keating, George and Johnson, 2015). Both of these benefits may mediate improvements in health-related quality of life (Gillison *et al.*, 2009; Awick *et al.*, 2015), whilst cardiorespiratory fitness remains a leading predictor of both cardiovascular and all-cause mortality (Blair *et al.*, 1989; Kodama *et al.*, 2009). This list is not exhaustive and an active lifestyle has several other benefits that are beyond the scope of discussion in this thesis. Notably, however, the potential psychosocial benefits of engaging in regular exercise and a physically active lifestyle should not be forgotten (Castellani *et al.*, 2003; Cadmus-Bertram *et al.*, 2014).

Exercise training also improves glycaemic control and much of this benefit may be mediated by improvements in insulin sensitivity (Garber *et al.*, 2011; Umpierre *et al.*, 2011; Fiuza-Luces *et al.*, 2013; Keating, George and Johnson, 2015). Insulin resistance is heavily implicated in

the pathogenesis of NAFLD, with consequential hyperglycaemia, hyperinsulinaemia and hyperlipidaemia all factors contributing to the development of hepatic steatosis (Fabbrini, Sullivan and Klein, 2010; Birkenfeld and Shulman, 2014; Brouwers *et al.*, 2016). However, insulin resistance is equally implicated in several other obesity-associated metabolic co-morbidities, including T2DM, which are common in patients with NAFLD (Armstrong *et al.*, 2014; Byrne and Targher, 2015). As such, improved insulin sensitivity and glycaemic control are clinically important outcomes for patients with NAFLD. The effects of exercise training on tissue-specific insulin sensitivity in patients with or at risk of NAFLD were explored in Chapters 6 and 7 of this thesis.

8.4 Insulin sensitivity

The study presented in Chapter 6 examined the effects of a 6-week SIT intervention on peripheral and hepatic insulin sensitivity in patients with NAFLD. Hepatic insulin sensitivity in both the basal and insulin-stimulated states was unaffected by SIT, but there was a tendency for improved peripheral insulin sensitivity. The latter is assumed to be primarily reflective of improved insulin sensitivity in skeletal muscle. Chapter 7 collated existing published literature exploring the effects of exercise training on HISI and $\%EGP_{supp}$ in patients with or at high risk of NAFLD. This included the data reported in Chapter 6. Despite limited evidence however, particularly with regards to the effects of training on $\%EGP_{supp}$, data pooling suggested that exercise training may improve HISI.

In Chapter 6, the response of peripheral insulin sensitivity to training was highly variable and ranged from a 59% increase in one individual to an 18% reduction in another (mean 18% improvement). Six individuals demonstrated an improvement (minimum 11%), whilst the remaining two showed a decline (11% and 18%, respectively). Several reasons may be responsible for this variability, including genetic factors and baseline characteristics (Böhm *et al.*, 2016). Furthermore, whilst (a) adherence to the training intervention was over 99%, (b) participants were instructed to make no changes to dietary habits and (c) no change in habitual physical activity was identified using hip-worn accelerometry, it cannot be discounted that variable responses may result from differences in participant motivation during training or compliance outside of the controlled environment. It should also be noted that the lack of response in some individuals may have been due to the short intervention duration and individuals showing no improvements after six weeks may have displayed an increase in peripheral insulin sensitivity had a longer intervention been utilised.

Improvements in peripheral insulin sensitivity will contribute to improved glucose tolerance and consequently lower postprandial hyperglycaemia and hyperinsulinaemia. In turn, lower circulating glucose and insulin may contribute to reductions in IHTG by reducing activation of ChREBP and SREBP-1c, respectively, thus lowering hepatic *DNL* (Yamashita *et al.*, 2001; Ameer *et al.*, 2014). Improved insulin sensitivity would also be of clinical benefit for many of the 22% of patients who have NAFLD and diabetes (particularly those with T2DM) (Younossi *et al.*, 2016) and may also reduce the risk of T2DM incidence in those without (Balkau *et al.*, 2010; Sung, Wild and Byrne, 2013; Mantovani *et al.*, 2018).

As mentioned previously, very few studies (n = 3) have investigated the effect of exercise training on hepatic insulin sensitivity in the insulin-stimulated state. Furthermore, this evidence remains contradictory. One study reported an improvement in $\%EGP_{supp}$ after six weeks of aerobic exercise training (Shojaee-Moradie *et al.*, 2007). However, it is notable that many of these individuals did not have NAFLD (median IHTG approximately 4%). The remaining two studies showed no effect of aerobic exercise or SIT on $\%EGP_{supp}$ (Cuthbertson *et al*. 2016; Sargeant *et al*. 2018; Chapter 6). The lack of studies exploring the effects of exercise on $\%EGP_{supp}$ may be the result, at least in part, of the technical expertise required to perform dual-step hyperinsulinaemic euglycaemic clamps with labelled glucose tracer, and the high financial cost. However, the results of the study in Chapter 6 and that of Cuthbertson *et al* (Cuthbertson *et al.*, 2016) are in accordance with existing evidence demonstrating that $\%EGP_{supp}$ may become substantially impaired at a very low level of IHTG; possibly as low as 1.5% (Bril, Barb, *et al.*, 2017). As such, in patients with NAFLD, a large reduction in IHTG, likely in association with substantial weight loss (see above), may be required to elicit improvements in insulin-stimulated hepatic insulin sensitivity.

Whilst the number of studies investigating the effects of exercise training on HISI is also limited, there was sufficient data to perform a small meta-analysis. This analysis demonstrated that, when data were pooled, exercise training elicits approximately 13% improvement in basal hepatic insulin sensitivity. This was despite very few studies reporting a statistically significant benefit within individual manuscripts. Notably, the HISI index is made up of two components, EGP and circulating insulin, and improvements with exercise training may be realised by reductions in the latter. Very few studies reported a reduction in basal rates of EGP after exercise training. This might be expected, however, given that in most of the studies participants were not hyperglycaemic. As such, improvements in basal hepatic insulin sensitivity may not result in lower rates of EGP because, in many individuals, these were

already normal. However, the concentration of circulating insulin required to maintain these rates, and thus fasted euglycaemia, may be reduced. These findings are supported by previous literature demonstrating that, in contrast to the relationship with $\%EGP_{supp}$, the relationship between IHTG and HISI may be linear (Korenblat *et al.*, 2008). Thus, any reduction in IHTG (such as the small reductions seen with exercise) may elicit improvements in basal hepatic insulin sensitivity.

8.5 Implementation of exercise training in the management of NAFLD

The findings of Chapters 6 and 7 demonstrate that exercise training has an important role in the management of NAFLD but its implementation alongside dietary energy restriction, with the aim of reducing total body weight, may be required to elicit the greatest benefits. Given that weight loss appears to be a critical modulator of change in IHTG, and dietary energy restriction has a greater capacity to induce large amounts of weight loss than exercise alone (Browning *et al.*, 2011; Wong *et al.*, 2013; Patel *et al.*, 2015), exercise training should not be considered a complete replacement to energy restriction for the majority of individuals. However, this does not mean that exercise training should be readily dismissed. Whilst the absolute effects may be smaller in comparison to energy restriction, the evidence from Chapters 6 and 7 of this thesis suggests that exercise training may contribute to reductions in IHTG. Identifying the precise mechanisms underlying these benefits was beyond the scope of this thesis but existing evidence suggests that both weight-dependent and independent effects may play a role (Brouwers *et al*., 2016). It is also important to remember that exercise training provides a wide array of benefits to individuals with NAFLD beyond effects on IHTG (Garber *et al*., 2011). Some of these benefits, which include improved glycaemic control and other components of cardiovascular disease risk, are discussed in more detail below.

Whilst the data presented in this thesis are novel, the promotion of a combined lifestyle intervention approach is not. In fact, the findings of this thesis support current guidelines for the management of NAFLD from the European Associations for the study of Liver (EASL), Diabetes (EASD) and Obesity (EASO), and the American Association for the Study of Liver Diseases (AASLD) (Marchesini *et al.*, 2016; Chalasani *et al.*, 2018). These guidelines endorse the promotion of structured exercise in conjunction with dietary energy restriction with the aim of reducing body weight by a minimum of 7-10%. Underpinning these guidelines is evidence that weight loss of at least 7% may be required to improve histological outcomes of NASH, in addition to reductions in IHTG (Promrat *et al.*, 2010; Vilar-Gomez *et al.*, 2015).

It is important to remember that, in similarity to pharmacological and surgical treatments, many forms of structured exercise exist and no 'one size fits all'. Consequently, there is the need to develop a variety of sustainable, evidence-based exercise interventions in order to provide health care professionals with a range of safe, effective options when formulating a treatment plan for a given individual. Importantly, with the ever-increasing focus on personalised medicine and prominence of patient preferences at the centre of treatment plans, having multiple potential exercise interventions also gives patients the chance to explore several different approaches throughout the ongoing management of their chronic condition. Data from Chapter 7, demonstrating that greater reductions in IHTG are elicited with interventions of longer duration, support the need to identify effective, sustainable exercise interventions.

The systematic review presented in Chapter 7 identified that aerobic and resistance exercise training, and several forms of HIIT (as well as combinations of two or more), have all been examined in individuals with NAFLD. Whilst the subgroup analysis performed in Chapter 7 is limited by the number of available studies, aerobic, HIIT and combined exercise training were each found to effectively reduce IHTG. These studies also include data presented in Chapter 6, where individuals with NAFLD were compliant with a SIT intervention involving repeated maximal exercise. However, the results of Chapter 7, along with previous reviews (Orci *et al.*, 2016), indicate that the greatest benefits on IHTG may be realised with aerobic exercise training and this may be mediated by a greater energy expenditure and subsequently greater potential for weight loss (Keating *et al.*, 2012; Yumuk *et al.*, 2015; Smart *et al.*, 2016).

Other studies (which did not utilise ^{1}H-MRS and therefore were not eligible for the review in Chapter 7) have shown that resistance exercise training may also reduce IHTG in patients with NAFLD (Bacchi *et al.*, 2013; Zelber-Sagi *et al.*, 2014). A recent review suggested that resistance exercise training may, in fact, be an important option for individuals with low cardiorespiratory fitness, as it may elicit a reduction in hepatic steatosis at a lower energy consumption (Hashida *et al.*, 2017). However, evidence for the direct benefits of resistance exercise training on hepatic outcomes in NAFLD remain inconclusive (Slentz *et al.*, 2011; Hickman *et al.*, 2013; Keating, George and Johnson, 2015). As previously discussed, however, patients with NAFLD will gain clinical benefits from factors beyond simply IHTG and resistance exercise may elicit many of these (Garber *et al.*, 2011; Fiuza-Luces *et al.*, 2013). Furthermore, evidence suggests that approximately one-third of weight loss may be attributed to the loss of metabolically and functionally important lean tissue mass (Heymsfield *et al.*, 2014; Weiss *et al.*, 2017). Resistance exercise training may attenuate this loss of lean mass and

help achieve a more favourable composition of weight loss (Weiss *et al.*, 2007; Villareal *et al.*, 2017). Resistance exercise has also been shown to elicit improvements in insulin sensitivity and glycaemic control (Garber *et al.*, 2011; Hallsworth *et al.*, 2011; Umpierre *et al.*, 2011; Strasser *et al.*, 2013).

Collectively, the findings in Chapters 6 and 7 of this thesis demonstrate that, where possible, the greatest benefits for individuals with NAFLD may be realised when structured exercise training is combined with dietary energy restriction with the aim of reducing body weight by at least 7%. However, studies directly comparing dietary energy restriction alone with combined diet and exercise training on IHTG, insulin sensitivity and other outcomes of clinical relevance in NAFLD would be valuable. Aerobic exercise is strongly endorsed as part of a structured exercise intervention to contribute to weight loss and improve cardiorespiratory fitness, whilst a resistance exercise component is firmly advised to induce wider metabolic benefits, promote the preservation of lean mass and improve physical function. Importantly, however, treatment approaches should be configured around patient preferences, motivations and individual circumstances to encourage sustainable changes in lifestyle behaviours. Furthermore, clinically relevant outcomes in NAFLD beyond IHTG and insulin sensitivity should also be considered.

8.6 Hepatokines

As mentioned previously, IHTG and insulin resistance are highly associated and the magnitude of IHTG is strongly predictive of insulin resistance not only in the liver, but also in skeletal muscle and adipose tissues (Korenblat *et al.*, 2008; Bril, Barb, *et al.*, 2017). Insulin resistance is heavily implicated in the pathogenesis of both NAFLD and T2DM and it is therefore unsurprising that these conditions commonly co-exist (Williamson *et al.*, 2011; Portillo Sanchez *et al.*, 2015; Cusi *et al.*, 2017). Individuals with both NAFLD and T2DM also have poorer prognosis (Vernon, Baranova and Younossi, 2011; Loomba *et al.*, 2012; Lomonaco *et al.*, 2016). In experimental rodent models, however, hepatic steatosis precedes the development of systemic insulin resistance during periods of high-fat overfeeding (Kraegen *et al.*, 1991; Davis *et al.*, 2010; Turner *et al.*, 2013) and in humans the presence of NAFLD is an independent predictor of T2DM incidence (Balkau *et al.*, 2010; Armstrong *et al.*, 2014; Mantovani *et al.*, 2018). This evidence suggests that the relationships between NAFLD and glucose regulation in peripheral tissues may not be simply associational, but changes in liver metabolism with the development of hepatic steatosis may act to modulate metabolic processes in the periphery

(Meex and Watt, 2017). Consequently, there is increasing interest into the potential mechanisms that may mediate cross-talk between the liver and peripheral tissues with the secretion of hepatokines proposed as one such mechanism (Stefan and Häring, 2013; Takamura, Misu and Kaneko, 2016; Meex and Watt, 2017).

Many hepatokines exist (Meex *et al.*, 2015), but five (FGF21, follistatin, fetuin-A, LECT2 and SeP) have been investigated in this thesis due to their reported influence on several aspects of glycaemic control (Misu *et al.*, 2010; Camporez *et al.*, 2013; Malin, Mulya, *et al.*, 2013; Lan *et al.*, 2014; Hansen, Rutti, *et al.*, 2016). Specifically, FGF21 and follistatin have positive actions by augmenting insulin signalling, increasing pancreatic β-cell proliferation or promoting the synthesis of skeletal muscle (Gilson *et al.*, 2009; Yaden *et al.*, 2014; Zhao *et al.*, 2015; Hansen, Rutti, *et al.*, 2016), whilst fetuin-A, LECT2 and SeP have deleterious effects on insulin signalling (Auberger *et al.*, 1989; Srinivas *et al.*, 1993; Misu *et al.*, 2012; Lan *et al.*, 2014).

Observational evidence in humans suggests that fasted concentrations of each of these hepatokines correlate with markers of insulin resistance, whilst all except follistatin correlate with adiposity (Chavez *et al.*, 2009; Brix *et al.*, 2010; Ishibashi *et al.*, 2010; Misu *et al.*, 2010; Yang, Hwang, *et al.*, 2011; Mashili *et al.*, 2011; Wu *et al.*, 2012; Ismail *et al.*, 2012; Ou, Yang, *et al.*, 2012; Hansen *et al.*, 2013; Okumura *et al.*, 2013; Lan *et al.*, 2014). Data presented in Chapter 4 of this thesis support these findings in part, with circulating concentrations of FGF21 and LECT2 reportedly higher in individuals with overweight or obesity (but normal glycaemic control), compared to normoglycaemic normal weight individuals. Furthermore, circulating FGF21 and LECT2 were positively correlated with markers of adiposity and insulin resistance, and negatively with cardiorespiratory fitness ($\dot{V}$O2 peak).

The studies presented in this thesis recruited individuals with a range of adiposity and glycaemic control. In addition to the normal weight, overweight and obese normoglycaemic individuals recruited in Chapter 4, the studies in Chapters 5 and 6 recruited overweight or obese individuals with dysglycaemia (HbA1c 5.7 to 6.5%) and NAFLD (who were also moderately insulin resistant), respectively. By pooling data from each these chapters, there is the opportunity to explore further the relationships between circulating hepatokine concentrations and markers of adiposity or insulin resistance in a more diverse population than that of any single chapter. Details of pooled data analysis can be found in Appendix XII.

The results of these pooled analyses support previous evidence, including that from Chapter 4, demonstrating that circulating concentrations of FGF21, follistatin and fetuin-A are elevated in association with increased adiposity and/or insulin resistance, as well as other markers of chronic metabolic disease. Interestingly, all of these hepatokines are upregulated in states of excess lipid storage or impaired glycaemic control. Given that FGF21 and follistatin have been shown to exert beneficial effects on glucose and lipid metabolism (Camporez *et al.*, 2013; Hansen, Rutti, *et al.*, 2016), these increased concentrations may represent adaptive responses in attempts to attenuate the decline in metabolic regulation. Conversely, fetuin-A has been shown to impair insulin signalling and may, therefore, be implicated in disease pathogenesis.

Pooled analyses also suggest that, despite correlating with indices of insulin resistance, fasted circulating concentrations of FGF21 may be influenced more by adiposity than glycaemic control. No differences were identified between overweight/obese individuals with normoglycaemia, dysglycaemia or NAFLD, but each of these groups had higher fasted plasma FGF21 than the normal weight individuals. This finding is supported by previous evidence demonstrating that, whilst FGF21 is elevated in individuals with T2DM, this may be the result of increased adiposity rather than impaired glycaemic control *per se* (Chavez *et al.*, 2009; Mraz *et al.*, 2009; Chen *et al.*, 2011; Mashili *et al.*, 2011). In contrast, follistatin and fetuin-A may only be elevated once a degree of metabolic dysregulation occurs as no differences were found between groups of normoglycaemic individuals irrespective of weight status. No previous study has identified independent relationships between markers of adiposity and circulating follistatin or fetuin-A. In fact, a study by Obuchi and colleagues found no difference in BMI between quartiles of fetuin-A in over 650 individuals (Obuchi *et al.*, 2014).

The primary aim of the studies in Chapters 4 and 5 of this thesis was to explore the effects of acute exercise on circulating hepatokine concentrations. These studies demonstrated that circulating concentrations of FGF21 and follistatin (but not LECT2, fetuin-A or SeP) are transiently elevated by a single bout of CME in both normal and overweight/obese individuals with normal glycaemic control (Chapter 4). However, only the follistatin response was maintained in individuals with dysglycaemia (Chapter 5), and a single bout of LV-HIIT did not affect circulating concentrations of any hepatokine measured in this thesis. Evidence suggests that the exercise response of FGF21 and follistatin to exercise may be mediated by changes in the glucagon to insulin ratio (Hansen *et al.*, 2015; Hansen, Pedersen, *et al.*, 2016; Hansen, Rutti, *et al.*, 2016) and this is supported by data in Chapter 4. This mechanism may also explain differential responses between different groups of individuals, such as those with normal or

impaired glycaemic control. Unfortunately, glucagon was not measured in Chapter 5 and, therefore, the glucagon to insulin ratio could not be determined. Other mechanisms have also been implicated in the regulation of hepatokines, such as hepatic AMPK (Okumura *et al.*, 2013; Lan *et al.*, 2014) and circulating NEFA (Badman *et al.*, 2007). In Chapter 4, circulating NEFA in the fasted state was higher in overweight/obese individuals, in accordance with higher FGF21 and LECT2. The response of circulating NEFA to acute exercise also mirrored that of FGF21. However, changes in systemic venous blood does not imply causality and more mechanistic studies containing the collection of hepatic and peripheral tissue samples and with isolation of the splanchnic circulation are required to directly investigate hepatokine regulation in humans.

Circulating concentrations of FGF21, follistatin, fetuin-A and LECT2 were also analysed before and after individuals with NAFLD completed a 6-week SIT intervention, but no changes were identified (Chapter 6). These findings, as well as the null findings in Chapters 4 and 5, may have been the results of a lack of statistical power (discussed in more detail below). However, it should also be noted that this is a novel area of research, particularly with regards to the effects of acute and/or chronic exercise on fetuin-A, LECT2 and SeP. No studies have previously investigated the effects of acute exercise on fetuin-A or SeP, whilst the single study investigating LECT2 was conducted in mice and utilised a particularly large volume of exercise (three hours of forced treadmill running) (Lan *et al.*, 2014). It could be, therefore, that hepatokine regulation differs between humans and rodent models or that the single bouts of exercise employed in Chapters 4 and 5 were simply insufficient to elicit a response.

Collectively, the data presented in this thesis support suggestions that circulating hepatokines may be altered with the development of chronic metabolic diseases such as NAFLD and T2DM, which are both associated with increased adiposity and an insulin-resistant state (Stefan and Häring, 2013; Takamura, Misu and Kaneko, 2016; Meex and Watt, 2017). Furthermore, some of these hepatokines (FGF21 and follistatin) may be modulated transiently by perturbations in energy metabolism such as a single bout of exercise. These hepatokines may, therefore, have a role in metabolic regulation, being secreted from the liver as a signal of metabolic stress (Hansen and Plomgaard, 2016). Given that FGF21 and follistatin also elicit favourable metabolic effects in peripheral tissues, repeated increases in these hepatokines with regular exercise may be implicated in mediating the metabolic benefits of an active lifestyle. However, further studies exploring the associations between hepatokines and chronic metabolic disease in clinical populations are required. Specifically, longitudinal studies examining changes in

circulating hepatokines over time with the development (or reversal) of metabolic dysfunction would be of interest. Furthermore, of specific relevance to the work conducted in this thesis, it would be interesting to explore whether the acute hepatokine response to a single bout of exercise changes with repeated bouts over an extended period (i.e. exercise training), and whether any changes in this response, or in fasted concentrations, are associated with improvements in metabolic parameters such as IHTG or insulin sensitivity.

It is important to acknowledge some limitations of the studies contained in this thesis. Firstly, the cross-sectional analyses presented in this thesis are exploratory and, as such, inferences remain speculative. Importantly, whilst the inclusion criteria for each experimental chapter of this thesis were varied in terms of weight status and glycaemic control, the pooled sample size remains small (n = 43) and most of these individuals were men (86%; n = 37). Moreover, all individuals recruited in this thesis were adults of white European ethnicity. Therefore, null findings may be the result of insufficient statistical power and these findings may not be generalizable to women, adolescents or children and different ethnic groups. Furthermore, the cross-sectional and correlational analyses presented in Chapter 4 and this General Discussion cannot be used to assess causality. Simple bivariate correlations were performed and many of the independent variables explored are strongly associated with one another. These analyses do not account for this shared variance and therefore the independent effects of the examined variables cannot be determined. Secondly, all hepatokine analyses conducted throughout this thesis were performed on plasma isolated from systemic venous blood. Cannulation of an antecubital vein is not optimal to assess changes in hepatokine secretion from the liver. Collection of systemic blood has been previously shown to be suitable to assess changes in FGF21 and follistatin after acute exercise (Hansen *et al.*, 2011; Hansen, Pedersen, *et al.*, 2016). However, the potential for changes in other circulating hepatokines (fetuin-A, LECT2 and SeP) to have been missed due to the location or timing of blood sampling cannot be dismissed. Finally, the hepatokine analyses presented may be limited by statistical power given that none of the studies reported in this thesis were formally powered to assess hepatokine outcomes. This may be particularly relevant for the data presented in Chapter 6. At the time of designing the studies in this thesis, there was very limited data available which investigated changes in circulating hepatokines with exercise. However, the data collected in this thesis may be used to inform future power calculations for larger studies in which changes in circulating hepatokines constitute the primary outcome.

Another interesting topic for discussion is that of adjusting for changes in plasma volume. In Chapter 4 of this thesis, the concentrations of all of our circulating biomarker outcomes (including hepatokines) were adjusted for changes in plasma volume; this was also the intention in Chapter 5 but large amounts of missing data for haematocrit and haemoglobin prevented this approach. The rationale for plasma volume adjustment during exercise studies comes from an observed haemoconcentration with exercise (Kargotich *et al*., 1998). As a result, changes in circulating concentrations of any given biomarker of interest may be due to changes in the plasma volume, rather than any change in biomarker production and secretion or clearance. This issue is, however, a widely debated one, with no definitive consensus currently apparent. One reasonable argument underlying the rationale not to adjust circulating biomarker concentrations, is that many homeostatic mechanisms appear to be based on the regulation of plasma biomarker concentration *per se*, regardless of any reason for change. For example, in individuals with normal glycaemic control, circulating glucose is regulated to a target concentration of approximately 4.5 $mmol \cdot L^{-1}$.

It is interesting to note that repeating statistical analyses using unadjusted data from the study in Chapter 4 has no impact on the results for FGF21, follistatin or fetuin-A. However, when using unadjusted data, significant interactions between trial and time are apparent for LECT2 ($P = 0.002$) and SeP ($P < 0.001$). In *post-hoc* tests LECT2 is significantly higher in the exercise trial at 1, 1.5 and 4 h (all $P \leq 0.03$), whilst SeP is significantly greater in the exercise trial at 1 h ($P< 0.001$) (Figure 8.1). Given that LECT2 and SeP are each associated with negative metabolic effects (Lan *et al*., 2014; Misu *et al*., 2010), an increase in these hepatokines after exercise is in contrast to that hypothesised in Chapter 4, and the physiological relevance or impact of these increased concentrations is currently unclear. The discrepancies between analyses performed on adjusted or unadjusted data do, however, highlight the important nature of this issue and the decision of which approach to take. In reality, the best approach may differ depending on any given biomarker of interest. When making this decision in future studies, researchers should consult the available literature to explore whether one approach or the other is more suitable in any given instance. If neither are, they may wish to present both adjusted and unadjusted data, or should at least be aware of the implications of the approach that they take.

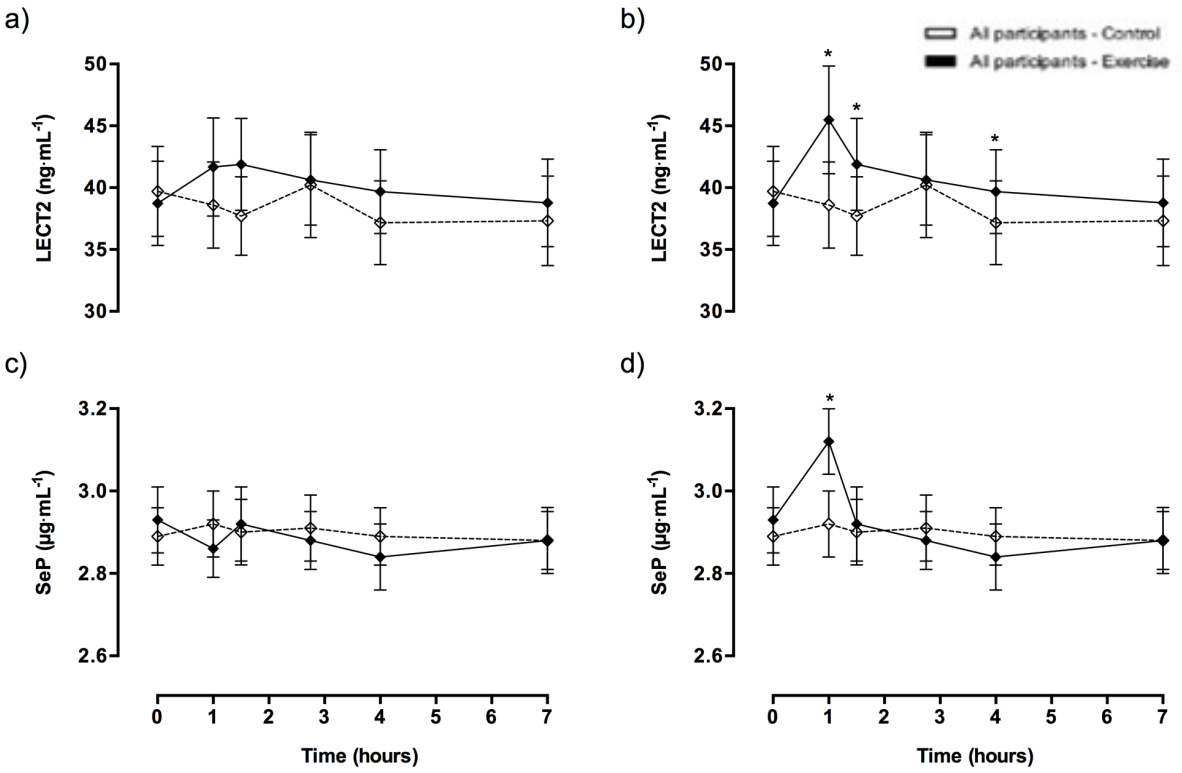


Figure 8.1 Circulating concentrations of LECT2 and SeP during control and exercise trials in the combined population of Chapter 4, using data adjusted (a & c) or unadjusted (b & d) for changes in plasma volume. Meals were provided at 1.5 and 4 h. Exercise was performed between 0 and 1 h in the exercise trial only. Data presented as mean ± SEM. * indicates significant difference from control trial at the same time point (all $P \leq 0.03$).

8.7 Future investigations

Whilst accepting their limitations, the findings in Chapters 4 and 5 provide sufficient rationale to explore the interaction between exercise (acute and chronic) and circulating hepatokines further. Investigating responses in different populations is warranted and it would be interesting to explore the relationship between changes in circulating hepatokines and changes in IHTG in patients with NAFLD. Furthermore, in addition to the long-term modulation of circulating hepatokines with the development or treatment of chronic metabolic disease, there is growing interest into their acute and short-term (days to weeks) regulation. Extending the findings presented in Chapter 4, the effect of exercise intensity on acute hepatokine responses is currently being explored within our research group (Willis *et al.*, 2018), as are the effects of other metabolic perturbations, such as short periods (1-7 d) of high-fat overfeeding (an abstract containing preliminary data has been submitted to BASES Conference 2018).

Whilst the findings of Chapters 6 and 7 are interesting, analyses are limited to the effects of exercise training on IHTG and hepatic or peripheral insulin sensitivity. The impact of exercise on other clinical outcomes relevant to the development and management of NAFLD (as well as associated metabolic co-morbidities) would also be welcomed. Specifically, studies exploring the effects of exercise training on hepatic inflammation and fibrosis would be particularly valuable (Keating and Adams, 2016), as the development of these hepatic complications (i.e. advanced NAFLD) is associated with poorer clinical prognosis (Ekstedt *et al.*, 2006; Söderberg *et al.*, 2010; D. Kim *et al.*, 2013; Younossi *et al.*, 2016). It is also possible to quantify indices of hepatic lipid quality (saturated vs unsaturated) using magnetic resonance procedures, which may be linked to a greater likelihood of developing hepatic insulin resistance (Johnson *et al.*, 2008). Furthermore, adipose tissue insulin sensitivity is heavily implicated in NAFLD, yet few studies have investigated the effects of exercise training on this outcome (Fabbrini *et al.*, 2008; Korenblat *et al.*, 2008; Lomonaco *et al.*, 2012; Bril, Barb, *et al.*, 2017).

Multiple studies, including that presented in Chapter 6, have demonstrated that individuals with NAFLD are able to complete various HIIT protocols in a controlled laboratory environment and these are associated with significant reductions in IHTG. This is particularly encouraging given that high-intensity exercise may be important in protecting individuals from the advanced NAFLD (Kistler *et al.*, 2011; Cho *et al.*, 2015). However, the development of high-intensity exercise interventions that are implementable in a clinical setting is essential if HIIT is to have any long-term clinical application in NAFLD.

CHAPTER 9

BIBLIOGRAPHY

Adams, L. A. (2013). NAFLD in the community. In G. C. Farrell, A. J. McCullough, & C. P. Day (Eds.), *Non-Alcoholic Fatty Liver Disease: A Practical Guide* (First, pp. 17–26). Wiley-Blackwell.

Adams, L. A., Lymp, J. F., St. Sauver, J., Sanderson, S. O., Lindor, K. D., Feldstein, A., & Angulo, P. (2005). The natural history of nonalcoholic fatty liver disease: A population-based cohort study. *Gastroenterology*, *129*(1), 113–121.

Adler, M., & Schaffner, F. (1979). Fatty liver hepatitis and cirrhosis in obese patients. *The American Journal of Medicine*, *67*(5), 811–816.

Aguilar-Olivos, N. E., Almeda-Valdes, P., Aguilar-Salinas, C. A., Uribe, M., & Méndez-Sánchez, N. (2016). The role of bariatric surgery in the management of nonalcoholic fatty liver disease and metabolic syndrome. *Metabolism: Clinical and Experimental*, *65*(8), 1–12.

Alam, S., Stolinski, M., Pentecost, C., Boroujerdi, M. A., Jones, R. H., Sonksen, P. H., & Umpleby, A. M. (2004). The effect of a six-month exercise program on very low-density lipoprotein apolipoprotein B secretion in type 2 diabetes. *The Journal of Clinical Endocrinology and Metabolism*, *89*(2), 688–694.

Aldred, H. E., Hardman, A. E., & Taylor, S. (1995). Influence of 12 weeks of training by brisk walking on postprandial lipemia and insulinemia in sedentary middle-aged women. *Metabolism*, *44*(3), 390–397.

Alkhouri, N., & Feldstein, A. E. (2016). Noninvasive diagnosis of nonalcoholic fatty liver disease: are we there yet? *Metabolism: Clinical and Experimental*, *65*(8), 1–9.

Altena, T., Michaelson, J., Ball, S., Guilford, B., & Thomas, T. (2006). Lipoprotein subfraction changes after continuous or intermittent exercise training. *Medicine & Science in Sports & Exercise*, *38*(2), 367–372.

Alves, T. C., Befroy, D. E., Kibbey, R. G., Kahn, M., Codella, R., Carvalho, R. A., … Shulman, G. I. (2011). Regulation of hepatic fat and glucose oxidation in rats with lipid-induced hepatic insulin resistance. *Hepatology*, *53*(4), 1175–1181.

Ameer, F., Scandiuzzi, L., Hasnain, S., Kalbacher, H., & Zaidi, N. (2014). De novo lipogenesis in health and disease. *Metabolism: Clinical and Experimental*, *63*(7), 895–902.

American Diabetes Association. (2018). Classification and diagnosis of diabetes: Standards of medical care in diabetes—2018. *Diabetes Care*, *41*(Supplement 1), S13–S27.

Andersen, G., Burgdorf, K. S., Sparsø, T., Borch-johnsen, K., Jørgensen, T., Hansen, T., & Pedersen, O. (2008). Studies of metabolic traits in 7,683 white Danish subjects. *Diabetes*, *57*(5), 1427–1432.

Angulo, P. (2010). Long-term mortality in nonalcoholic fatty liver disease: Is liver histology of any prognostic significance? *Hepatology*, *51*(2), 373–375.

Annuzzi, G., De Natale, C., Iovine, C., Patti, L., Di Marino, L., Coppola, S., … Rivellese, A. A. (2004). Insulin resistance is independently associated with postprandial alterations of triglyceride-rich lipoproteins in type 2 diabetes mellitus. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *24*(12), 2397–2402.

Anson, M., Crain-Denoyelle, A. M., Baud, V., Chereau, F., Gougelet, A., Terris, B., … Couty, J. P. (2012). Oncogenic β-catenin triggers an inflammatory response that determines the aggressiveness of hepatocellular carcinoma in mice. *Journal of Clinical Investigation*, *122*(2), 586–599.

Anstee, Q. M., Targher, G., & Day, C. P. (2013). Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nature Reviews Gastroenterology & Hepatology*, *10*(6), 330–344.

Argo, C. K., Patrie, J. T., Lackner, C., Henry, T. D., De Lange, E. E., Weltman, A. L., … Caldwell, S. H. (2015). Effects of n-3 fish oil on metabolic and histological parameters in NASH: A double-blind, randomized, placebo-controlled trial. *Journal of Hepatology*, *62*(1), 190–197.

Armstrong, M. J., Adams, L. A., Canbay, A., & Syn, W.-K. (2014). Extrahepatic complications of nonalcoholic fatty liver disease. *Hepatology*, *59*(3), 1174–1197.

Armstrong, M. J., Gaunt, P., Aithal, G. P., Barton, D., Hull, D., Parker, R., … Newsome, P. N. (2016). Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): A multicentre, double-blind, randomised, placebo-controlled phase 2 study. *The Lancet*, *387*, 679–690.

Armstrong, M. J., Houlihan, D. D., Bentham, L., Shaw, J. C., Cramb, R., Olliff, S., … Newsome, P. N. (2012). Presence and severity of non-alcoholic fatty liver disease in a large prospective primary care cohort. *Journal of Hepatology*, *56*(1), 234–240.

Artunc, F., Schleicher, E., Weigert, C., Fritsche, A., Stefan, N., & Haring, H.-U. (2016). The impact of insulin resistance on the kidney and vasculature. *Nature Review Nephrology*, *12*, 721–737.

Assy, N., Kaita, K., Mymin, D., Levy, C., Rosser, B., & Minuk, G. (2000). Fatty infiltration of liver in hyperlipidemic patients. *Digestive Diseases and Sciences*, *45*(10), 1929–1934.

Astorino, T. A., Allen, R. P., Roberson, D. W., & Jurancich, M. (2012). Effect of high-intensity interval training on cardiovascular function, $\dot{V}$O2max, and muscular force. *Journal of Strength and Conditioning Research*, *26*(1), 138–145.

Atkinson, G. (2001). Analysis of repeated measurements in physical therapy research. *Physical Therapy in Sport*, *2*(4), 194–208.

Attar, B. M., & Van Thiel, D. H. (2013). Current concepts and management approaches in nonalcoholic fatty liver disease. *The Scientific World Journal*, *2013*, 1-10.

Auberger, P., Falquerho, L., Contreres, J. O., Pages, G., Le Cam, G., Rossi, B., & Le Cam, A. (1989). Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. *Cell*, *58*(4), 631–640.

Awick, E. A., Wójcicki, T. R., Olson, E. A., Fanning, J., Chung, H. D., Zuniga, K., … McAuley, E. (2015). Differential exercise effects on quality of life and health-related quality of life in older adults: a randomized controlled trial. *Quality of Life Research*, *24*(2), 455–462.

Babraj, J. A., Vollaard, N. B. J., Keast, C., Guppy, F. M., Cottrell, G., & Timmons, J. A. (2009). Extremely short duration high intensity interval training substantially improves insulin action in young healthy males. *BMC Endocrine Disorders*, *9(3)*, 1-8.

Bacchi, E., Negri, C., Targher, G., Faccioli, N., Lanza, M., Zoppini, G., … Moghetti, P. (2013). Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 Randomized Trial). *Hepatology*, *58*(4), 1287–1295.

Badman, M. K., Koester, A., Flier, J. S., Kharitonenkov, A., & Maratos-Flier, E. (2009). Fibroblast growth factor 21-deficient mice demonstrate impaired adaptation to ketosis. *Endocrinology*, *150*(11), 4931–4940.

Badman, M. K., Pissios, P., Kennedy, A. R., Koukos, G., Flier, J. S., & Maratos-Flier, E. (2007). Hepatic fibroblast growth factor 21 is regulated by PPARα and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell Metabolism*, *5*(6), 426–437.

Baldry, E., Aithal, G., Kaye, P., Idris, I., Bennett, A., Leeder, P., & Macdonald, I. (2017). Effects of short-term energy restriction on liver lipid content and inflammatory status in severely obese adults: results of a randomised controlled trial (RCT) using two dietary approaches. *Diabetes, Obesity & Metabolism*, 1–23.

Balkau, B., Lange, C., Vol, S., Fumeron, F., & Bonnet, F. (2010). Nine-year incident diabetes is predicted by fatty liver indices:the French D.E.S.I.R study. *BMC Gastroenterology*, *10*(56), 1–9.

Bar-Or, O. (1987). The wingate anaerobic test. *Sports Medicine*, *4*(6), 381–394.

Bawden, S., Scott, R., & Aithal, G. (2017). Current and future magnetic resonance technologies for assessing liver disease in clinical and experimental medicine. *Digestive Diseases*, *35*(4), 314–322.

Berglund, E. D., Li, C. Y., Bina, H. A., Lynes, S. E., Michael, M. D., Shanafelt, A. B., … Wasserman, D. H. (2009). Fibroblast growth factor 21 controls glycemia via regulation of hepatic glucose flux and insulin sensitivity. *Endocrinology*, *150*(9), 4084–4093.

Besse-Patin, A., Montastier, E., Vinel, C., Castan-Laurell, I., Louche, K., Dray, C., … Viguerie, N. (2014). Effect of endurance training on skeletal muscle myokine expression in obese men: identification of apelin as a novel myokine. *International Journal of Obesity*, *38*(5), 707–713.

Birkenfeld, A. L., & Shulman, G. I. (2014). Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 Diabetes. *Hepatology*, *59*(2), 713–723.

Blair, S., Kohl III, H., Paffenbarger Jr, R., Clark, D., Cooper, K., & Gibbons, L. (1989). Physical fitness and all-cause mortality. *Journal of the American Medical Association*, *262*(17), 2395–2401.

Blais, P., Husain, N., Kramer, J. R., Kowalkowski, M., El-Serag, H., & Kanwal, F. (2015). Nonalcoholic fatty liver disease is underrecognized in the primary care setting. *The American Journal of Gastroenterology*, *110*(1), 10–14.

Blüher, M. (2013). Adipose tissue dysfunction contributes to obesity related metabolic diseases. *Best Practice & Research Clinical Endocrinology & Metabolism*, *27*(2), 163–177.

Blüher, M., Rudich, A., Klöting, N., Golan, R., Henkin, Y., Rubin, E., … Shai, I. (2012). Two patterns of adipokine and other biomarker dynamics in a long-term weight loss intervention. *Diabetes Care*, *35*, 342–349.

Bobbert, T., Schwarz, F., Fischer-Rosinsky, A., Pfeiffer, A. F. H., Möhlig, M., Mai, K., & Spranger, J. (2013). Fibroblast growth factor 21 predicts the metabolic syndrome and type 2 diabetes in Caucasians. *Diabetes Care*, *36*(1), 145–149.

Boden, G. (2008). Obesity and free fatty acids (FFAs). *Endocrinology and Metabolism Clinics of North America*, *37*(3), 1–12.

Böhm, A., Weigert, C., Staiger, H., & Häring, H.-U. (2016). Exercise and diabetes: relevance and causes for response variability. *Endocrine*, *51*(3), 390–401.

Borg, G. (1970). Perceived exertion as an indicator of somatic stress. *Scandinavian Journal of Rehabilitation Medicine*, *2*(2), 92–98.

Borjesson, M., Urhausen, A., Kouidi, E., Dugmore, D., Sharma, S., Halle, M., … Vanhees, L. (2011). Cardiovascular evaluation of middle-aged/senior individuals engaged in leisure-time sport activities: Position stand from the sections of exercise physiology and sports cardiology of the European Association of Cardiovascular Prevention and Rehabilitation. *European Journal of Cardiovascular Prevention and Rehabilitation*, *18*(3), 446–458.

Bradbury, M. W. (2006). Hepatic fatty acid uptake: Possible role in steatosis. *American Journal of Physiology Gastrointestinal & Liver Physiology*, *290*(12), 194–198.

Bray, G. A. (2004). Medical consequences of obesity. *The Journal of Clinical Endocrinology and Metabolism*, *89*(6), 2583–2589.

Bray, G. A., Frühbeck, G., Ryan, D. H., & Wilding, J. P. H. (2016). Management of obesity. *The Lancet*, *387*, 1947–1956.

Brea, A., & Puzo, J. (2013). Non-alcoholic fatty liver disease and cardiovascular risk. *International Journal of Cardiology*, *167*(4), 1109–1117.

Bril, F., Barb, D., Portillo-Sanchez, P., Biernacki, D., Lomonaco, R., Suman, A., … Cusi, K. (2017). Metabolic and histological implications of intrahepatic triglyceride content in nonalcoholic fatty liver disease. *Hepatology*, *65*(4), 1132–1144.

Bril, F., & Cusi, K. (2017). Management of nonalcoholic fatty liver disease in patients with type 2 diabetes: A call to action. *Diabetes Care*, *40*(3), 419–430.

Bril, F., Portillo-Sanchez, P., Liu, I.-C., Kalavalapalli, S., Dayton, K., & Cusi, K. (2017). Clinical and histologic characterization of nonalcoholic steatohepatitis in African American patients. *Diabetes Care*, ePub ahead of print.

Brix, J. M., Stingl, H., Höllerl, F., Schernthaner, G. H., Kopp, H.-P., & Schernthaner, G. (2010). Elevated fetuin-A concentrations in morbid obesity decrease after dramatic weight loss. *The Journal of Clinical Endocrinology and Metabolism*, *95*(11), 4877–4881.

Brouwers, B., Hesselink, M. K. C., Schrauwen, P., & Schrauwen-Hinderling, V. B. (2016). Effects of exercise training on intrahepatic lipid content in humans. *Diabetologia*, *59*(10), 2068–2079.

Browning, J. D., Baker, J. A., Rogers, T., Davis, J., Satapati, S., & Burgess, S. C. (2011). Short-term weight loss and hepatic triglyceride reduction: Evidence of a metabolic advantage with dietary carbohydrate restriction. *American Journal of Clinical Nutrition*, *93*(5), 1048–1052.

Browning, J. D., Szczepaniak, L. S., Dobbins, R., Nuremberg, P., Horton, J. D., Cohen, J. C., … Hobbs, H. H. (2004). Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. *Hepatology*, *40*(6), 1387–1395.

Burgomaster, K. A., Howarth, K. R., Phillips, S. M., Rakobowchuk, M., Macdonald, M. J., McGee, S. L., & Gibala, M. J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *The Journal of Physiology*, *586*(1), 151–160.

Burgomaster, K. A, Hughes, S. C., Heigenhauser, G. J. F., Bradwell, S. N., & Gibala, M. J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *Journal of Applied Physiology*, *98*(6), 1985–1990.

Burk, R. F., & Hill, K. E. (2005). Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. *Annual Review of Nutrition*, *25*, 215–235.

Burk, R. F., & Hill, K. E. (2009). Selenoprotein P – Expression, functions, and roles in mammals. *Biochimica et Biophysica Acta*, *1790*(11), 1441–1447.

Buzzetti, E., Pinzani, M., & Tsochatzis, E. A. (2016). The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism: Clinical and Experimental*, *65*(8), 1–11.

Byrne, C. D. (2013). Ectopic fat, insulin resistance and non-alcoholic fatty liver disease. *The Proceedings of the Nutrition Society*, *72*(4), 412–419.

Byrne, C. D., & Targher, G. (2014). Ectopic fat, insulin resistance, and nonalcoholic fatty liver disease: implications for cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *34*(6), 1155–1161.

Byrne, C. D., & Targher, G. (2015). NAFLD: A multisystem disease. *Journal of Hepatology*, *62*(1), S47–S64.

Cadmus-Bertram, L., Irwin, M., Alfano, C., Campbell, K., Foster-Schubert, K., Wang, C. Y., & McTiernan, A. (2014). Predicting adherence of adults to a 12-month exercise intervention. *Journal of Physical Activity and Health*, *11*(7), 1304–1312.

Cai, D., Yuan, M., Frantz, D. F., Melendez, P. A., Hansen, L., Lee, J., & Shoelson, S. E. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK- β and NF- κ B, *11*(2), 183–190.

Caldwell, S., & Argo, C. (2010). The natural history of non-alcoholic fatty liver disease. *Digestive Diseases*, *28*(1), 162–168.

Camacho, R. C., Donahue, E. P., James, F. D., Berglund, E. D., Wasserman, D. H., Berglund, D., & Wasserman, D. H. (2006). Energy state of the liver during short-term and exhaustive exercise in C57BL/6J mice. *American Journal of Physiology Endocrinology and Metabolism*, *290*(3), 405–408.

Camporez, J. P. G., Jornayvaz, F. R., Petersen, M. C., Pesta, D., Guigni, B. A., Serr, J., … Shulman, G. I. (2013). Cellular mechanisms by which FGF21 improves insulin sensitivity in male mice. *Endocrinology*, *154*(9), 3099–3109.

Cassader, M., Gambino, R., Musso, G., Depetris, N., Mecca, F., Cavallo-Perin, P., … Pagano, G. (2001). Postprandial triglyceride-rich lipoprotein metabolism and insulin sensitivity in nonalcoholic steatohepatitis patients. *Lipids*, *36*(10), 1117–1124.

Cassidy, S., Thoma, C., Hallsworth, K., Parikh, J., Hollingsworth, K. G., Taylor, R., … Trenell, M. I. (2016). High intensity intermittent exercise improves cardiac structure and function and reduces liver fat in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia*, *59*(1), 56–66.

Castellani, W., Ianni, L., Ricca, V., Mannucci, E., & Rotella, C. M. (2003). Adherence to structured physical exercise in overweight and obese subjects: A review of psychological models. *Eating and Weight Disorders*, *8*(1), 1–11.

Chakravarthy, M. V., Pan, Z., Zhu, Y., Tordjman, K., Schneider, J. G., Coleman, T., … Semenkovich, C. F. (2005). "New" hepatic fat activates PPARα to maintain glucose, lipid, and cholesterol homeostasis. *Cell Metabolism*, *1*(5), 309–322.

Chalasani, N., Christopher Gorski, J., Asghar, M. S., Asghar, A., Foresman, B., Hall, S. D., & Crabb, D. W. (2003). Hepatic cytochrome P450 2E1 activity in nondiabetic patients with nonalcoholic steatohepatitis. *Hepatology*, *37*(3), 544–550.

Chalasani, N., Younossi, Z., Lavine, J. E., Charlton, M., Cusi, K., Rinella, M., … Sanyal, A. J. (2018). The diagnosis and management of non-alcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*, *67*(1), 328–357.

Charatcharoenwitthaya, P., Lindor, K. D., & Angulo, P. (2012). The spontaneous course of liver enzymes and its correlation in nonalcoholic fatty liver disease. *Digestive Diseases and Sciences*, *57*(7), 1925–1931.

Chavez, A. O., Molina-Carrion, M., Abdul-Ghani, M. A., Folli, F., DeFronzo, R. A., & Tripathy, D. (2009). Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. *Diabetes Care*, *32*(8), 1542–1546.

Chen, C., Cheung, B. M. Y., Tso, A. W. K., Wang, Y., Law, L. S. C., Ong, K. L., … Lam, K. S. L. (2011). High plasma level of fibroblast growth factor 21 is an independent predictor of type 2 diabetes: A 5.4-year population-based prospective study in Chinese subjects. *Diabetes Care*, *34*(9), 2113–2115.

Chikamoto, K., Misu, H., Takayama, H., Kikuchi, A., Ishii, K. A., Lan, F., … Takamura, T. (2016). Rapid response of the steatosis-sensing hepatokine LECT2 during diet-induced weight cycling in mice. *Biochemical and Biophysical Research Communications*, *478*(3), 1310–1316.

Cho, J., Kim, S., Lee, S., & Kang, H. (2015). Effect of training intensity on nonalcoholic fatty liver disease. *Medicine & Science in Sports & Exercise*, *47*(8), 1624–1634.

Choi, H. Y., Hwang, S. Y., Lee, C. H., Hong, H. C., Yang, S. J., Yoo, H. J., … Choi, K. M. (2013). Increased selenoprotein P levels in subjects with visceral obesity and nonalcoholic fatty liver disease. *Diabetes and Metabolism Journal*, *37*(1), 63–71.

Choi, K. M., Han, K. A., Ahn, H. J., Lee, S. Y., Hwang, S. Y., Kim, B. H., … Min, K. W. (2013). The effects of caloric restriction on Fetuin-A and cardiovascular risk factors in rats and humans: A randomized controlled trial. *Clinical Endocrinology*, *79*(3), 356–363.

Church, T. S., Thomas, D. M., Tudor-Locke, C., Katzmarzyk, P. T., Earnest, C. P., Rodarte, R. Q., … Bouchard, C. (2011). Trends over 5 decades in U.S. occupation-related physical activity and their associations with obesity. *PLoS ONE*, *6*(5), 1–7.

Cocks, M., Shaw, C. S., Shepherd, S. O., Fisher, J. P., Ranasinghe, A., Barker, T. A., & Wagenmakers, A. J. M. (2015). Sprint interval and moderate-intensity continuous training have equal benefits on aerobic capacity, insulin sensitivity, muscle capillarisation and endothelial eNOS/NAD(P)Hoxidase protein ratio in obese men. *The Journal of Physiology*, 1–15.

Cocks, M., Shaw, C. S., Shepherd, S. O., Fisher, J. P., Ranasinghe, A. M., Barker, T. A., … Wagenmakers, A. J. M. (2013). Sprint interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males. *Journal of Physiology*, *591*(3), 641–656.

Cohen, J. (1988). *Statistical power analysis for the behavioural sciences* (2nd ed.). Hillsdale, NJ: Lawrence Earlbaum Associates.

Cohen, J., Noakes, T., & Benade, A. (1989). Postprandial lipemia and chylomicron clearance in athletes and in sedentary men. *American Journal of Clinical Nutrition*, *49*, 443–447.

Coker, R. H., Hays, N. P., Williams, R. H., Brown, A. D., Freeling, S. A., Kortebein, P. M., … Evans, W. J. (2006). Exercise-induced changes in insulin action and glycogen metabolism in elderly adults. *Medicine and Science in Sports and Exercise*, *38*(3), 433–438.

Coker, R. H., Williams, R. H., Yeo, S. E., Kortebein, P. M., Bodenner, D. L., Kern, P. A, & Evans, W. J. (2009). The impact of exercise training compared to caloric restriction on hepatic and peripheral insulin resistance in obesity. *The Journal of Clinical Endocrinology and Metabolism*, *94*(11), 4258–4266.

Colberg, S. R., Sigal, R. J., Yardley, J. E., Riddell, M. C., Dunstan, D. W., Dempsey, P. C., … Tate, D. F. (2016). Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. *Diabetes Care*, *39*(11), 2065–2079.

Cook, J. R., Langlet, F., Kido, Y., & Accili, D. (2015). Pathogenesis of selective insulin resistance in isolated hepatocytes. *Journal of Biological Chemistry*, *290*(22), 13972–13980.

Coquart, J., Lemaire, C., Dubart, A.-E., Luttembacher, D.-P., Douillard, C., & Garcin, M. (2008). Intermittent versus continuous exercise: Effects of perceptually lower exercise in obese women. *Medicine and Science in Sports and Exercise*, *40*(8), 1546–1553.

Coskun, T., Bina, H. A., Schneider, M. A., Dunbar, J. D., Hu, C. C., Chen, Y., … Kharitonenkov, A. (2008). Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology*, *149*(12), 6018–6027.

Cuevas-Ramos, D., Aguilar-Salinas, C. A., & Gómez-Pérez, F. J. (2012). Metabolic actions of fibroblast growth factor 21. *Current Opinion in Pediatrics*, *24*(4), 523–529.

Cuevas-Ramos, D., Almeda-Valdes, P., Gómez-Pérez, F. J., Meza-Arana, C. E., Cruz-Bautista, I., Arellano-Campos, O., … Aguilar-Salinas, C. A. (2010). Daily physical activity, fasting glucose, uric acid, and body mass index are independent factors associated with serum fibroblast growth factor 21 levels. *European Journal of Endocrinology*, *163*(3), 469–477.

Cuevas-Ramos, D., Almeda-Valdés, P., Meza-Arana, C. E., Brito-Córdova, G., Gómez-Pérez, F. J., Mehta, R., … Aguilar-Salinas, C. A. (2012). Exercise increases serum fibroblast growth factor 21 (FGF21) levels. *PloS One*, *7*(5), e38022.

Currie, K. D., Dubberley, J. B., McKelvie, R. S., & Macdonald, M. J. (2013). Low-volume, high-intensity interval training in patients with CAD. *Medicine and Science in Sports and Exercise*, *45*(8), 1436–1442.

Cusi, K., Consoli, A., & DeFronzo, R. (1996). Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology & Metabolism*, *81*(11), 4059–4067.

Cusi, K., Sanyal, A., Zhang, S., Hartman, M., Bue-Valleskey, J., Hoogwerf, B., & Haupt, A. (2017). Non-alcoholic fatty liver disease (NAFLD) prevalence and its metabolic associations in patients with type 1 diabetes and type 2 diabetes. *Diabetes, Obesity & Metabolism*, ePub ahead of print.

Cuthbertson, D. J., Shojaee-Moradie, F., Sprung, V. S., Jones, H., Pugh, C. J. A., Richardson, P., … Umpleby, A. M. (2016). Dissociation between exercise-induced reduction in liver fat and changes in hepatic and peripheral glucose homoeostasis in obese patients with non-alcoholic fatty liver disease. *Clinical Science*, *130*(2), 93–104.

Dasarathy, S., Dasarathy, J., Khiyami, A., Yerian, L., Hawkins, C., Sargent, R., & McCullough, A. (2015). Double blind randomized placebo controlled clinical trial of omega 3 fatty acids for the treatment of diabetic patients with nonalcoholic steatohepatitis. *Journal of Clinical Gastroenterology*, *49*(2), 133–144.

Dasgupta, S., Bhattacharya, S., Biswas, A., Majumdar, S. S., Mukhopadhyay, S., Ray, S., & Bhattacharya, S. (2010). NF-κB mediates lipid-induced fetuin-A expression in hepatocytes that impairs adipocyte function effecting insulin resistance. *The Biochemical Journal*, *429*(3), 451–462.

Davis, R. C., Castellani, L. W., Hosseini, M., Ben-zeev, O., Mao, H. Z., Weinstein, M. M., … Lusis, A. J. (2010). Early hepatic insulin resistance precedes the onset of diabetes in obese C57BLKS-db / db mice. *Diabetes*, *59*(7).

DeFronzo, R. A., Simonson, D., & Ferrannini, E. (1982). Hepatic and peripheral insulin resistance: A common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*, *23*(4), 313–319.

DeFronzo, R. A., Tobin, J. D., & Andres, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *American Journal of Physiology*, *237*(3), E214–E223.

DeFronzo, R., Sherwin, R., & Kraemer, N. (1987). The effect of physical training on insulin action in obesity. *Diabetes*, *36*, 1379–1385.

Deighton, K., Barry, R., Connon, C. E., & Stensel, D. J. (2013). Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *European Journal of Applied Physiology*, *113*(5), 1147–1156.

Denecke, B., Graber, S., Chafer, C., Heiss, A., Woltje, M., Jahnen-Dechent, W., … Jahnen-Dechent, W. (2003). Tissue distribution and activity testing suggest a similar but not identical function of fetuin-B and fetuin-A. *The Biochemical Journal*, *376*, 135–145.

Dhurandhar, N. V, Schoeller, D., Brown, A. W., Heymsfield, S. B., Thomas, D., Sørensen, T. I. A., … Allison, D. B. (2015). Energy balance measurement: when something is not better than nothing. *International Journal of Obesity*, *39*(7), 1109–1113.

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, *37*(2), 247–248.

DiPietro, L., Dziura, J., Yeckel, C. W., & Neufer, P. D. (2006). Exercise and improved insulin sensitivity in older women: evidence of the enduring benefits of higher intensity training. *Journal of Applied Physiology*, *100*(1), 142–149.

Diraison, F., Moulin, P., & Beylot, M. (2003). Contribution of hepatic de novo lipogenesis and reesertification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metabolism Journal*, *29*, 478–485.

Dongiovanni, P., Petta, S., Maglio, C., Fracanzani, A. L., Pipitone, R., Mozzi, E., … Valenti, L. (2015). Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology*, *61*(2), 506–514.

Dongiovanni, P., Petta, S., Mannisto, V., Mancina, R. M., Pipitone, R., Karja, V., … Valenti, L. (2015). Statin use and non-alcoholic steatohepatitis in at risk individuals. *Journal of Hepatology*, *63*(3), 705–712.

Donnelly, K. L., Smith, C. I., Schwarzenberg, S. J., Jessurun, J., Boldt, M. D., & Parks, E. J. (2005). Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *Journal of Clinical Investigation*, *115*(5), 1343–1351.

Douglas, J. A., King, J. A., Clayton, D. J., Jackson, A. P., Sargeant, J. A., Thackray, A. E., … Stensel, D. J. (2017). Acute effects of exercise on appetite, ad libitum energy intake and appetite-regulatory hormones in lean and overweight/obese men and women. *International Journal of Obesity*, *41*(12), 1737–1744.

Downs, S. H., & Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *Journal of Epidemiology & Community Health*, *52*(6), 377–384.

Dushay, J., Chui, P. C., Gopalakrishnan, G. S., Varela-Rey, M., Crawley, M., Fisher, F. M., … Maratos-Flier, E. (2010). Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. *Gastroenterology*, *139*(2), 456–463.

Dutta, D., Mondal, S. A., Kumar, M., Hasanoor Reza, A. H., Biswas, D., Singh, P., … Mukhopadhyay, S. (2014). Serum fetuin-A concentration predicts glycaemic outcomes in people with prediabetes: A prospective study from eastern India. *Diabetic Medicine*, *31*(12), 1594–1599.

Ekstedt, M., Franzén, L. E., Mathiesen, U. L., Thorelius, L., Holmqvist, M., Bodemar, G., & Kechagias, S. (2006). Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*, *44*(4), 865–873.

Fabbrini, E., & Magkos, F. (2015). Hepatic steatosis as a marker of metabolic dysfunction. *Nutrients*, *7*, 4995–5019.

Fabbrini, E., Magkos, F., Mohammed, B. S., Pietka, T., Abumrad, N. A., Patterson, B. W., … Klein, S. (2009). Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proceedings of the National Academy of Sciences U S A*, *106*(36), 15430–15435.

Fabbrini, E., Mohammed, B. S., Magkos, F., Korenblat, K. M., Patterson, B. W., & Klein, S. (2008). Alterations in adipose tissue and hepatic lipid kinetics on obese men and women with nonalcoholic fatty liver disease. *Gastroenterology*, *134*(2), 424–431.

Fabbrini, E., Sullivan, S., & Klein, S. (2010). Obesity and nonalcoholic fatty liver disease: Biochemical, metabolic, and clinical implications. *Hepatology*, *51*(2), 679–689.

FAO, WHO, & UNU. (2001). *Human energy requirements: Report of a joint FAO/WHO/UNU expert consultation*. Food & Agriculture Organisation. Retrieved from http://www.fao.org/docrep/007/y5686e/y5686e00.htm

Festi, D., Schiumerini, R., Marzi, L., Di Biase, A. R., Mandolesi, D., Montrone, L., … Colecchia, A. (2013). Review article: the diagnosis of non-alcoholic fatty liver disease -- availability and accuracy of non-invasive methods. *Alimentary Pharmacology & Therapeutics*, *37*(4), 392–400.

Fisher, M., Chui, P. C., Antonellis, P. J., Bina, H. A., Kharitonenkov, A., Flier, J. S., & Maratos-flier, E. (2010). Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. *Diabetes*, *59*(11), 2781–2789.

Fiuza-Luces, C., Garatachea, N., Berger, N. A., & Lucia, A. (2013). Exercise is the real polypill. *Physiology*, *28*, 330–358.

Flannery, C., Dufour, S., Rabøl, R., Shulman, G. I., & Petersen, K. F. (2012). Skeletal muscle insulin resistance promotes increased hepatic de novo lipogenesis, hyperlipidemia, and hepatic steatosis in the elderly. *Diabetes*, *61*(11), 2711–2717.

Fon Tacer, K., Bookout, A. L., Ding, X., Kurosu, H., John, G. B., Wang, L., … Kliewer, S. A. (2010). Comprehensive expression atlas of the fibroblast growth factor system in adult mouse. *Molecular Endocrinology*, *24*(10), 2050–2064.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, *18*(6), 499–502.

Frith, J., Day, C. P., Henderson, E., Burt, A. D., & Newton, J. L. (2009). Non-alcoholic fatty liver disease in older people. *Gerontology*, *55*(6), 607–613.

Frühbeck, G., Méndez-Giménez, L., Fernández-Formoso, J. A., Fernández, S., & Rodríguez, A. (2014). Regulation of adipocyte lipolysis. *Nutrition Research Reviews 27*(1), 63-93.

Gaggini, M., Morelli, M., Buzzigoli, E., DeFronzo, R. A., Bugianesi, E., & Gastaldelli, A. (2013). Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. *Nutrients*, *5*(5), 1544–1560.

Gaich, G., Chien, J. Y., Fu, H., Glass, L. C., Deeg, M. A., Holland, W. L., … Moller, D. E. (2013). The effects of LY2405319, an FGF21 Analog, in obese human subjects with type 2 diabetes. *Cell Metabolism*, *18*(3), 333–340.

Gälman, C., Lundåsen, T., Kharitonenkov, A., Bina, H. A., Eriksson, M., Hafström, I., … Rudling, M. (2008). The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARα activation in man. *Cell Metabolism*, *8*(2), 169–174.

Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A, Lamonte, M. J., Lee, I.-M., … Swain, D. P. (2011). American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Medicine and Science in Sports and Exercise*, *43*(7), 1334–1359.

Gastaldelli, A., Cusi, K., Pettiti, M., Hardies, J., Miyazaki, Y., Berria, R., … Defronzo, R. A. (2007). Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology*, *133*(2), 496–506.

Gazala, S., Horwitz, E., Ben-Haroush Schyr, R., Bardugo, A., Israeli, H., Hija, A., … Ben-Zvi, D. (2018). Sleeve gastrectomy improves glycemia independent of weight loss by restoring hepatic insulin sensitivity. *Diabetes*, ePub ahead of print.

Gibala, M. J., Gillen, J. B., & Percival, M. E. (2014). Physiological and health-related adaptations to low-volume interval training: influences of nutrition and sex. *Sports Medicine*, *44 (Suppl 2)*, 127–137.

Gibala, M. J., Little, J. P., Macdonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *The Journal of Physiology*, *590*(5), 1077–1084.

Gibala, M. J., Little, J. P., van Essen, M., Wilkin, G. P., Burgomaster, K. A., Safdar, A., … Tarnopolsky, M. A. (2006). Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *The Journal of Physiology*, *575*(3), 901–911.

Gill, J. M. R. (2004). Exercise and postprandial lipid metabolism - An analysis of the current evidence. *European Journal of Lipid Science and Technology*, *106*(2), 110–121.

Gillen, J. B., Percival, M. E., Ludzki, A., Tarnopolsky, M. A., & Gibala, M. J. (2013). Interval training in the fed or fasted state improves body composition and muscle oxidative capacity in overweight women. *Obesity*, *21*(11), 2249–2255.

Gillen, J., Little, J., Punthakee, Z., Tarnopolsky, M., Riddell, M., & Gibala, M. (2012). Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism*, *14*, 575–577.

Gillison, F. B., Skevington, S. M., Sato, A., Standage, M., & Evangelidou, S. (2009). The effects of exercise interventions on quality of life in clinical and healthy populations; a meta-analysis. *Social Science and Medicine*, *68*(9), 1700–1710.

Gilson, H., Schakman, O., Kalista, S., Lause, P., Tsuchida, K., & Thissen, J.-P. (2009). Follistatin induces muscle hypertrophy through satellite cell proliferation and inhibition of both myostatin and activin. *American Journal of Physiology. Endocrinology and Metabolism*, *297*(1), E157–E164.

Gimeno, R. E., & Moller, D. E. (2014). FGF21-based pharmacotherapy - potential utility for metabolic disorders. *Trends in Endocrinology and Metabolism*, *25*(6), 303–311.

Gist, N. H., Fedewa, M. V, Dishman, R. K., & Cureton, K. J. (2014). Sprint interval training effects on aerobic capacity: a systematic review and meta-analysis. *Sports Medicine, 44*(2), 269–279.

Golabi, P., Locklear, C. T., Austin, P., Afdhal, S., Byrns, M., Gerber, L., & Younossi, Z. M. (2016). Effectiveness of exercise in hepatic fat mobilization in nonalcoholic fatty liver disease: Systematic review. *World Journal of Gastroenterology*, *22*(27), 6318–6327.

Goldberg, I. J., Eckel, R. H., & Abumrad, N. A. (2009). Regulation of fatty acid uptake into tissues: lipoprotein lipase- and CD36-mediated pathways: *Journal of Lipid Research*, *50*, S86–S90.

Goustin, A. S., Derar, N., & Abou-Samra, A. B. (2013). Ahsg-fetuin blocks the metabolic arm of insulin action through its interaction with the 95-kD β-subunit of the insulin receptor. *Cellular Signalling*, *25*(4), 981–988.

Greco, D., Kotronen, A., Westerbacka, J., Puig, O., Arkkila, P., Kiviluoto, T., … Auvinen, P. (2008). Gene expression in human NAFLD. *American Journal of Physiology Gastrointestinal and Liver Physiology*, *294*, 1281–1287.

Green, C. J., & Hodson, L. (2014). The influence of dietary fat on liver fat accumulation. *Nutrients*, *6*(11), 5018–5033.

Guiraud, T., Nigam, A., Juneau, M., Meyer, P., Gayda, M., & Bosquet, L. (2011). Acute responses to high-intensity intermittent exercise in CHD patients. *Medicine and Science in Sports and Exercise*, *43*(2), 211–217.

Guth, E. (2014). Healthy weight loss. *Journal of the Amercian Medical Association, 312*(9), 974.

Hallsworth, K., Fattakhova, G., Hollingsworth, K. G., Thoma, C., Moore, S., Taylor, R., … Trenell, M. I. (2011). Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut*, *60*(9), 1278–1283.

Hallsworth, K., Thoma, C., Hollingsworth, K. G., Cassidy, S., Anstee, Q. M., Day, C. P., & Trenell, M. I. (2015). Modified high-intensity interval training reduces liver fat and improves cardiac function in non-alcoholic fatty liver disease: a randomized controlled trial. *Clinical Science*, *129*(12), 1097–1105.

Hannukainen, J. C., Nuutila, P., Borra, R., Ronald, B., Kaprio, J., Kujala, U. M., … Kalliokoski, K. K. (2007). Increased physical activity decreases hepatic free fatty acid uptake: a study in human monozygotic twins. *The Journal of Physiology*, *578*(1), 347–358.

Hansen, J., Brandt, C., Nielsen, A. R., Hojman, P., Whitham, M., Febbraio, M. A, … Plomgaard, P. (2011). Exercise induces a marked increase in plasma follistatin: evidence that follistatin is a contraction-induced hepatokine. *Endocrinology*, *152*(1), 164–171.

Hansen, J., Clemmesen, J., Secher, N., Hoene, M., Drescher, A., Weigert, C., … Plomgaard, P. (2015). Glucagon-to-insulin ratio is pivotal for splanchnic regulation of FGF-21 in humans. *Molecular Metabolism*, *4*(8), 551–560.

Hansen, J., Pedersen, B. K., Xu, G., Lehmann, R., Weigert, C., & Plomgaard, P. (2016). Exercise-induced secretion of FGF21 and follistatin are blocked by pancreatic clamp and impaired in type 2 diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *101*(7), 2816–2825.

Hansen, J., & Plomgaard, P. (2016). Circulating follistatin in relation to energy metabolism. *Molecular and Cellular Endocrinology*, *433*, 87–93.

Hansen, J., Rinnov, A., Krogh-Madsen, R., Fischer, C. P., Andreasen, A. S., Berg, R. M. G., … Plomgaard, P. (2013). Plasma follistatin is elevated in patients with type 2 diabetes: relationship to hyperglycemia, hyperinsulinemia and systemic low-grade inflammation. *Diabetes/metabolism Research and Reviews*, *29*(6), 463–472.

Hansen, J., Rutti, S., Arous, C., Clemmesen, J. O., Secher, N. H., Drescher, A., … Plomgaard, P. (2016). Circulating follistatin is liver-derived and regulated by the glucagon-to-insulin ratio. *The Journal of Clinical Endocrinology & Metabolism*, *101*(2), 550–560.

Harrison, S. A., Fecht, W., Brunt, E. M., & Neuschwander-Tetri, B. A. (2009). Orlistat for overweight subjects with nonalcoholic steatohepatitis: A randomized, prospective trial. *Hepatology*, *49*(1), 80–86.

Hashida, R., Kawaguchi, T., Bekki, M., Omoto, M., Matsuse, H., Nago, T., … Torimura, T. (2017). Aerobic versus resistance exercise in non-alcoholic fatty liver disease: A systematic review. *Journal of Hepatology*, *66*(1), 142–152.

Haufe, S., Engeli, S., Kast, P., Böhnke, J., Utz, W., Haas, V., … Jordan, J. (2011). Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. *Hepatology*, *53*(5), 1504–1514.

Haukeland, J. W., Dahl, T. B., Yndestad, A., Gladhaug, I. P., Løberg, E. M., Haaland, T., … Birkeland, K. I. (2012). Fetuin A in nonalcoholic fatty liver disease: in vivo and in vitro studies. *European Journal of Endocrinology*, *166*(3), 503–510.

Haukeland, J. W., Konopski, Z., Eggesbø, H. B., Von Volkmann, H. L., Raschpichler, G., Bjøro, K., … Birkeland, K. (2009). Metformin in patients with non-alcoholic fatty liver disease: A randomized, controlled trial. *Scandinavian Journal of Gastroenterology*, *44*(7), 853–860.

Haus, J. M., Solomon, T. P. J., Kelly, K. R., Fealy, C. E., Kullman, E. L., Scelsi, A. R., … Kirwan, J. P. (2013). Improved hepatic lipid composition following short-term exercise in nonalcoholic fatty liver disease. *The Journal of Clinical Endocrinology and Metabolism*, *98*(7), E1181-1188.

Hazell, T. J., MacPherson, R. E. K., Gravelle, B. M. R., & Lemon, P. W. R. (2010). 10 or 30-s sprint interval training bouts enhance both aerobic and anaerobic performance. *European Journal of Applied Physiology*, *110*(1), 153–160.

Heisz, J. J., Tejada, M. G. M., Paolucci, E. M., & Muir, C. (2016). Enjoyment for high-intensity interval exercise increases during the first six weeks of training: Implications for promoting exercise adherence in sedentary adults. *Plos One*, *11*(12), e0168534.

Hennige, A. M., Staiger, H., Wicke, C., Machicao, F., Fritsche, A., Häring, H.-U., & Stefan, N. (2008). Fetuin-A induces cytokine expression and suppresses adiponectin production. *PloS One*, *3*(3), e1765.

Herd, S. L., Hardman, A. E., Boobis, L. H., & Cairns, C. J. (1998). The effect of 13 weeks of running training followed by 9 d of detraining on postprandial lipaemia. *The British Journal of Nutrition*, *80*(1), 57–66.

Heymsfield, S. B., Gonzalez, M. C. C., Shen, W., Redman, L., & Thomas, D. (2014). Weight loss composition is one-fourth fat-free mass: A critical review and critique of this widely cited rule. *Obesity Reviews*, *15*(4), 310–321.

Heymsfield, S. B., & Wadden, T. A. (2017). Mechanisms, pathophysiology, and management of obesity. *New England Journal of Medicine*, *376*(3), 254–266.

Hickman, I., Byrne, N., Croci, I., Chachay, V., Clouston, A., Hills, A., … Macdonald, G. (2013). A pilot randomised study of the metabolic and histological effects of exercise in non-alcoholic steatohepatitis. *Journal of Diabetes & Metabolism*, *4*(8), 300.

Higgins, J., & Green, S. (2011). *Cochrance Handbook for Systematic Reviews of Interventions*. (J. Higgins & S. Green, Eds.), *The Cochrane Collaboration*. Chichester: John Wiley & Sons Ltd. Retrieved from www.cochrane-handbook.org

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, *6*(2), 65–70.

Holt, H. B., Wild, S. H., Wood, P. J., Zhang, J., Darekar, A. A., Dewbury, K., … Byrne, C. D. (2006). Non-esterified fatty acid concentrations are independently associated with hepatic steatosis in obese subjects. *Diabetologia*, *49*(1), 141–148.

Hood, M. S., Little, J. P., Tarnopolsky, M. a., Myslik, F., & Gibala, M. J. (2011). Low-volume interval training improves muscle oxidative capacity in sedentary adults. *Medicine and Science in Sports and Exercise*, *43*(10), 1849–1856.

Hotamisligil, G. S. (2010). Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell*, *140*(6), 900–917.

Houghton, D., Thoma, C., Hallsworth, K., Cassidy, S., Burt, A. D., Tiniakos, D., … Trenell, M. I. (2017). Exercise reduces liver lipids and visceral adiposity in patients with non-alcoholic steatohepatitis in a randomized controlled trial. *Clinical Gastroenterology and Hepatology*, *15*(1), 96–102.

Hughes, V. A, Fiatarone, M. A, Fielding, R. A, Kahn, B. B., Ferrara, C. M., Shepherd, P., … Evans, W. J. (1993). Exercise increases muscle GLUT-4 levels and insulin action in subjects with impaired glucose tolerance. *The American Journal of Physiology*, *264*(6), E855–E862.

Hussey, S. E., Lum, H., Alvarez, A., Cipriani, Y., Garduno-Garcia, J., Anaya, L., … Musi, N. (2014). A sustained increase in plasma NEFA upregulates the toll-like receptor network in human muscle. *Diabetologia*, *57*(3), 582–591.

Hwang, H.-J., Jung, T. W., Kim, B.-H., Hong, H. C., Seo, J. A., Kim, S. G., … Yoo, H. J. (2015). A dipeptidyl peptidase-IV inhibitor improves hepatic steatosis and insulin resistance by AMPK-dependent and JNK-dependent inhibition of LECT2 expression. *Biochemical Pharmacology*, *98*(1), 157–166.

Iizuka, K., Takeda, J., & Horikawa, Y. (2009). Glucose induces FGF21 mRNA expression through ChREBP activation in rat hepatocytes. *FEBS Letters*, *583*(17), 2882–2886.

Iozzo, P., Bucci, M., Roivainen, A., Någren, K., Järvisalo, M. J., Kiss, J., … Nuutila, P. (2010). Fatty acid metabolism in the liver, measured by positron emission tomography, is increased in obese individuals. *Gastroenterology*, *139*(3), 846–856.

Iozzo, P., Takala, T., Oikonen, V., Bergman, J., Gronroos, T., Ferrannini, E., … Knuuti, J. (2004). Effect of training status on regional disposal of circulating free fatty acids in the liver and skeletal muscle during physiological hyperinsulinemia. *Diabetes Care*, *27*, 2172–2177.

Ishibashi, A., Ikeda, Y., Ohguro, T., Kumon, Y., Yamanaka, S., Takata, H., … Terada, Y. (2010). Serum fetuin-A is an independent marker of insulin resistance in Japanese men. *Journal of Atherosclerosis and Thrombosis*, *17*(9), 925–933.

Ismail, N. A., Ragab, S., Abd El Dayem, S. M., Abd ElBaky, A., Salah, N., Hamed, M., … Koura, H. (2012). Fetuin-A levels in obesity: Differences in relation to metabolic syndrome and correlation with clinical and laboratory variables. *Archives of Medical Science*, *8*(5), 826–833.

Ix, J. H., Biggs, M. L., Mukamal, K. J., Kizer, J. R., Zieman, S. J., Siscovick, D. S., … Djousse, L. (2012). Association of fetuin-A with incident diabetes mellitus in community-living older adults: The cardiovascular health study. *Circulation*, *125*(19), 2316–2322.

Ix, J. H., Shlipak, M. G., Brandenburg, V. M., Ali, S., Ketteler, M., & Whooley, M. A. (2006). Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. *Circulation*, *113*(14), 1760–1767.

Jacobs, R. A., Fluck, D., Bonne, T. C., Burgi, S., Christensen, P. M., Toigo, M., & Lundby, C. (2013). Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial content and function. *Journal of Applied Physiology*, *115*(6), 785–793.

Jacome-Sosa, M. M., & Parks, E. J. (2014). Fatty acid sources and their fluxes as they contribute to plasma triglyceride concentrations and fatty liver in humans. *Current Opinion in Lipidology*, *25*(3), 213–220.

James, P. A., Oparil, S., Carter, B. L., Cushman, W. C., Dennison-Himmelfarb, C., Handler, J., … Ortiz, E. (2014). 2014 Evidence-based guidelines for the management of high blood pressure in adults. *Journal of the Amercian Medical Association*, *311*(5), 507–520.

Jelenik, T., Kaul, K., Séquaris, G., Flögel, U., Phielix, E., Kotzka, J., … Roden, M. (2017). Mechanisms of insulin resistance in primary and secondary non-alcoholic fatty liver. *Diabetes*, *66*(8), 2241-2253.

Jenkins, N. T., Mckenzie, J. A., Hagberg, J. M., & Witkowski, S. (2011). Plasma fetuin-A concentrations in young and older high- and low-active men. *Metabolism: Clinical and Experimental*, *60*(2), 265–271.

Jensen, M. D., Ryan, D. H., Apovian, C. M., Ard, J. D., Comuzzie, A. G., Donato, K. A., … Yanovski, S. Z. (2014). 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: A report of the American College of cardiology/American Heart Association task force on practice guidelines and the obesity society. *Circulation*, *129*(Suppl 2), S102–S138.

Jensen, T., Abdelmalek, M. F., Sullivan, S., Nadeau, K. J., Green, M., Roncal, C., … Johnson, R. J. (2018). Fructose and Sugar: A Major Mediator of Nonalcoholic Fatty Liver Disease. *Journal of Hepatology*. ePub ahead of print.

Jensky, N. E., Sims, J. K., Rice, J. C., Dreyer, H. C., & Schroeder, E. T. (2007). The influence of eccentric exercise on mRNA expression of skeletal muscle regulators. *European Journal of Applied Physiology*, *101*(4), 473–480.

Jensky, N., Sims, J., Dieli-conwright, C., Sattler, F., Rice, J., & Schroeder, E. (2010). Exercise does not influence myostatin and follistatin mRNA expression in young women. *Journal of Strength and Conditioning Research*, *24*(2), 522-589.

Johnson, N. A., Sachinwalla, T., Walton, D. W., Smith, K., Armstrong, A., Thompson, M. W., & George, J. (2009). Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology*, *50*(4), 1105–1112.

Johnson, N. A., Walton, D. W., Sachinwalla, T., Thompson, C. H., Smith, K., Ruell, P. A., … George, J. (2008). Noninvasive assessment of hepatic lipid composition: Advancing understanding and management of fatty liver disorders. *Hepatology, 47*(5), 1513–1523.

Johnston, R. D., Stephenson, M. C., Crossland, H., Cordon, S. M., Palcidi, E., Cox, E. F., … Macdonald, I. A. (2013). No difference between high-fructose and high-glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. *Gastroenterology*, *145*(5), 1016–1025.

Jornayvaz, F. R., & Shulman, G. I. (2012). Diacylglycerol activation of protein kinase Cε and hepatic insulin resistance. *Cell Metabolism*, *15*(5), 574–584.

Jung, M. E., Bourne, J. E., & Little, J. P. (2014). Where does HIT fit? An examination of the affective response to high-intensity intervals in comparison to continuous moderate- and continuous vigorous-intensity exercise in the exercise intensity-affect continuum. *PloS One*, *9*(12), 1-18.

Jung, T. W., Choi, H. Y., Lee, S. Y., Hong, H. C., Yang, S. J., Yoo, H. J., … Choi, K. M. (2013). Salsalate and adiponectin improve palmitate-induced insulin resistance via inhibition of selenoprotein P through the AMPK-FOXO1α pathway. *PLoS ONE*, *8*(6), 1–9.

Jung, T. W., Yoo, H. J., & Choi, K. M. (2016). Implication of hepatokines in metabolic disorders and cardiovascular diseases. *BBA Clinical*, *5*, 108–113.

Jung, T. W., Youn, B.-S., Choi, H. Y., Lee, S. Y., Hong, H. C., Yang, S. J., … Choi, K. M. (2013). Salsalate and adiponectin ameliorate hepatic steatosis by inhibition of the hepatokine fetuin-A. *Biochemical Pharmacology*, *86*(7), 960–969.

Kaess, B. M., Enserro, D. M., McManus, D. D., Xanthakis, V., Chen, M. H., Sullivan, L. M., … Glazer, N. L. (2012). Cardiometabolic correlates and heritability of fetuin-A, retinol-binding protein 4, and fatty-acid binding protein 4 in the framingham heart study. *Journal of Clinical Endocrinology and Metabolism*, *97*(10), 1943–1947.

Kantartzis, K., MacHann, J., Schick, F., Fritsche, A., Häring, H.-U., & Stefan, N. (2010). The impact of liver fat vs visceral fat in determining categories of prediabetes. *Diabetologia*, *53*(5), 882–889.

Kargotich, S., Goodman, C., Keast, D., & Morton, A. R. (1998). The influence of exercise-induced plasma volume changes on the interpretation of biochemical parameters used for monitoring exercise, training and sport. *Sports Medicine, 26*(2), 101-117.

Karpe, F., & Pinnick, K. (2015). Biology of upper-body and lower-body adipose tissue - link to whole-body phenotypes. *Nature Reviews Endocrinology*, *11*(2), 90–100.

Katsagoni, C. N., Georgoulis, M., Papatheodoridis, G. V., Panagiotakos, D. B., & Kontogianni, M. D. (2017). Effects of lifestyle interventions on clinical characteristics of patients with non-alcoholic fatty liver disease: A meta-analysis. *Metabolism: Clinical and Experimental*, *68*, 119–132.

Keating, S. E., & Adams, L. A. (2016). Exercise in NAFLD: Just do it. *Journal of Hepatology*, *65*, 671–673.

Keating, S., George, J., & Johnson, N. (2015). The benefits of exercise for patients with non-alcoholic fatty liver disease. *Expert Review of Gastroenterology & Hepatology*, *9*(10), 1247–1250.

Keating, S., Hackett, D., George, J., & Johnson, N. (2012). Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Journal of Hepatology*, *57*(1), 157–166.

Keating, S., Hackett, D., Parker, H., O'Connor, H., Gerofi, J., Sainsbury, A., … Johnson, N. (2015). Effect of aerobic exercise training dose on liver fat and visceral adiposity. *Journal of Hepatology*, *63*(1), 174–182.

Kelly, S., Martin, S., Kuhn, I., Cowan, A., Brayne, C., & Lafortune, L. (2016). Barriers and facilitators to the uptake and maintenance of healthy behaviours by people at mid-life: A rapid systematic review. *PLoS ONE*, *11*(1), 1–26.

Kelly, T., Yang, W., Chen, C.-S., Reynolds, K., & He, J. (2008). Global burden of obesity in 2005 and projections to 2030. *International Journal of Obesity*, *32*(9), 1431–1437.

Kessler, H. S., Sisson, S. B., & Short, K. R. (2012). The potential for high-intensity interval training to reduce cardiometabolic disease risk. *Sports Medicine*, *42*(6), 489–509.

Kharitonenkov, A., & Adams, A. C. (2014). Inventing new medicines: The FGF21 story. *Molecular Metabolism*, *3*(3), 221–229.

Kharitonenkov, A., Shiyanova, T. L., Koester, A., Ford, A. M., Micanovic, R., Galbreath, E. J., … Shanafelt, A. B. (2005). FGF-21 as a novel metabolic regulator. *Journal of Clinical Investigation*, *115*(6), 1627–1635.

Kilpatrick, M. W., Martinez, N., Little, J. P., Jung, M. E., Jones, A. M., Price, N. W., & Lende, D. H. (2015). Impact of high-intensity interval duration on perceived exertion. *Medicine and Science in Sports and Exercise*, *47(5)*, 1038-1045.

Kim, D., Kim, W. R., Kim, H. J., & Therneau, T. M. (2013). Association between non-invasive fibrosis markers and mortality among adults with non-alcoholic fatty liver disease in the United States. *Hepatology*, *57*(4), 1357–1365.

Kim, I.-Y., Suh, S.-H., Lee, I.-K., & Wolfe, R. R. (2016). Applications of stable, nonradioactive isotope tracers in in vivo human metabolic research. *Experimental & Molecular Medicine*, *48*(12), e203.

Kim, K. H., Kim, S. H., Min, Y.-K., Yang, H.-M., Lee, J.-B., & Lee, M.-S. (2013). Acute exercise induces FGF21 expression in mice and in healthy humans. *PloS One*, *8*(5), e63517.

Kim, S. H., Kim, K. H., Kim, H.-K., Kim, M.-J., Back, S. H., Konishi, M., … Lee, M.-S. (2015). Fibroblast growth factor 21 participates in adaptation to endoplasmic reticulum stress and attenuates obesity-induced hepatic metabolic stress. *Diabetologia*, 809–818.

Kirk, E., Reeds, D. N., Finck, B. N., Mayurranjan, S. M., Mayurranjan, M. S., Patterson, B. W., & Klein, S. (2009). Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. *Gastroenterology*, *136*(5), 1552–1560.

Kirk, E., Sullivan, S., & Klein, S. (2010). Exercise reduces intrahepatic triglyceride and improves insulin action in adults with nonalcoholic fatty liver disease. *Medicine & Science in Sports & Exercise*, *42*(5), 44.

Kistler, K. D., Brunt, E. M., Clark, J. M., Mae, A., Sallis, J. F., Schwimmer, J. B., & NASH CRN Research Group. (2011). Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *American Journal of Gastroenterology*, *106*(3), 460–468.

Klöting, N., Fasshauer, M., Dietrich, A., Kovacs, P., Schön, M. R., Kern, M., … Blüher, M. (2010). Insulin-sensitive obesity. *American Journal of Physiology. Endocrinology and Metabolism*, *299*(3), E506–E515.

Kodama, S., Saito, K., Tanaka, S., Maki, M., Yachi, Y., Asumi, M., … Sone, H. (2009). Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: A meta-analysis. *Journal of the American Medical Association*, *301*(19), 2024–2035.

Kolak, M., Westerbacka, J., Velagapudi, V., Wågsater, D., Yetukuri, L., Makkonen, J., … Yki-Järvinen, H. (2007). Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. *Diabetes*, *56*(8), 1960–1968.

Koliaki, C., Szendroedi, J., Kaul, K., Jelenik, T., Nowotny, P., Jankowiak, F., … Roden, M. (2015). Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metabolism*, *21*(5), 739–746.

Korenblat, K. M., Fabbrini, E., Mohammed, B. S., & Klein, S. (2008). Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. *Gastroenterology*, *134*(5), 1369–1375.

Kotani, K., Peroni, O., Minokoshi, Y., Boss, O., & Kahn, B. (2004). GLUT4 glucose transporter deficiency increases hepatic lipid production and peripheral lipid util ... *Journal of Clinical Investigation*, *114*(11), 1666–1675.

Kotronen, A., & Yki-Järvinen, H. (2008). Fatty liver: a novel component of the metabolic syndrome. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *28*(1), 27–38.

Kraegen, E. W., Clark, P. W., Jenkins, A. B., Daley, E. A., Chisholm, D. J., & Storlien, L. H. (1991). Development of muscle insulin resistance after liver insulin resistance in high fat-fed rats. *Diabetes*, *40*, 1397–1403.

Kumashiro, N., Erion, D. M., Zhang, D., Kahn, M., Beddow, S. A., Chu, X., … Shulman, G. I. (2011). Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(39), 16381–16385.

Laakso, M., & Kuusisto, J. (2014). Insulin resistance and hyperglycaemia in cardiovascular disease development. *Nature Reviews Endocrinology*, *10*(5), 293–302.

Lambert, J. E., Ramos-Roman, M. A., Browning, J. D., & Parks, E. J. (2014). Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology*, *146*(3), 726–735.

Lan, F., Misu, H., Chikamoto, K., Takayama, H., Kikuchi, A., Mohri, K., … Takamura, T. (2014). LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance. *Diabetes*, *63*(5), 1649–1664.

Langleite, T. M., Jensen, J., Norheim, F., Gulseth, H. L., Tangen, D. S., Kolnes, K. J., … Drevon, C. A. (2016). Insulin sensitivity, body composition and adipose depots following 12 w combined endurance and strength training in dysglycemic and normoglycemic sedentary men. *Archives of Physiology and Biochemistry*, *122*(4), 167–179.

Lassailly, G., Caiazzo, R., Buob, D., Pigeyre, M., Verkindt, H., Labreuche, J., … Mathurin, P. (2015). Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. *Gastroenterology*, *149*(2), 379–388.

Lavine, J., Schwimmer, J., Van Natta, M., Molleston, J., Murray, K., Rosenthal, P., … Robuck, P. (2011). Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents. *Journal of the Amercian Medical Association*, *305*(16), 1659–1668.

Lazo, M., Solga, S. F., Horska, A., Bonekamp, S., Diehl, A. M., Brancati, F. L., … Clark, J. M. (2010). Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. *Diabetes Care*, *33*(10), 2156–2163.

Lebensztejn, D. M., Flisiak-Jackiewicz, M., Białokoz-Kalinowska, I., Bobrus-Chociej, A., & Kowalska, I. (2016). Hepatokines and non-alcoholic fatty liver disease. *ACTA ABP Biochimica Polonica*, *63*(3), 459-467.

Leclerq, I. A. (2013). Emerging concepts on the pathogenesis of nonalcoholic steatohepatitis (NASH). In G. C. Farrell, A. J. McCullough, & C. P. Day (Eds.), *Non-Alcoholic Fatty Liver Disease: A Practical Guide* (First Edit, pp. 46–61). Chichester.

Lecoultre, V., Egli, L., Carrel, G., Theytaz, F., Kreis, R., Schneiter, P., … Tappy, L. (2013). Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. *Obesity*, *21*(4), 782–785.

Lee, S.-J., & McPherron, A. C. (2001). Regulation of myostatin activity and muscle growth. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(16), 9306–9311.

Lee, S., Deldin, A. R., White, D., Kim, Y., Libman, I., Rivera-Vega, M., … Arslanian, S. (2013). Aerobic exercise but not resistance exercise reduces intrahepatic lipid content and visceral fat and improves insulin sensitivity in obese adolescent girls: a randomized controlled trial. *American Journal of Physiology. Endocrinology and Metabolism*, *305*(10), E1222-12229.

Leslie, M. (2015). The liver's weighty problem. *Science*, *349*(6433), 18–20.

Li, H., Fang, Q., Gao, F., Fan, J., Zhou, J., Wang, X., … Jia, W. (2010). Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. *Journal of Hepatology*, *53*(5), 934–940.

Li, H., Wu, G., Fang, Q., Zhang, M., Hui, X., Sheng, B., … Jia, W. (2018). Fibroblast growth factor 21 increases insulin sensitivity through specific expansion of subcutaneous fat. *Nature Communications, 9*(272), 1-10.

Lin, X., Braymer, H. D., Bray, G. A., & York, D. A. (1998). Differential expression of insulin receptor tyrosine kinase inhibitor (fetuin) gene in a model of diet-induced obesity. *Life Sciences*, *63*(2), 145–153.

Linden, M. A., Fletcher, J. A., Morris, E. M., Meers, G. M., Kearney, M. L., Crissey, J. M., … Rector, R. S. (2014). Combining metformin and aerobic exercise training in the treatment of type 2 diabetes and NAFLD in OLETF rats. *American Journal of Physiology. Endocrinology Metabolism*, *306*(3), E300-10.

Linden, M. A., Fletcher, J. A., Morris, E. M., Meers, G. M., Laughlin, M. H., Booth, F. W., … Rector, R. S. (2015). Treating NAFLD in OLETF rats with vigorous-intensity interval exercise training. *Medicine and Science in Sports and Exercise*, *47*(3), 556–567.

Little, J. P., Gillen, J. B., Percival, M. E., Safdar, A., Tarnopolsky, M. A, Punthakee, Z., … Gibala, M. J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *Journal of Applied Physiology*, *111*(6), 1554–1560.

Little, J. P., Jung, M. E., Wright, A. E., Wright, W., & Manders, R. J. F. (2014). Effects of high-intensity interval exercise versus continuous moderate-intensity exercise on postprandial glycemic control assessed by continuous glucose monitoring in obese adults. *Applied Physiology, Nutrition, and Metabolism*, *39*(7), 835–841.

Little, J. P., Safdar, A., Wilkin, G. P., Tarnopolsky, M. A, & Gibala, M. J. (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *The Journal of Physiology*, *588*(6), 1011–1022.

Lomonaco, R., Bril, F., Portillo-Sanchez, P., Ortiz-Lopez, C., Orsak, B., Biernacki, D., … Cusi, K. (2016). Metabolic impact of nonalcoholic steatohepatitis in obese patients with type 2 diabetes. *Diabetes Care*, *39*(4), 632-648.

Lomonaco, R., Ortiz-Lopez, C., Orsak, B., Webb, A., Hardies, J., Darland, C., … Cusi, K. (2012). Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. *Hepatology*, *55*(5), 1389–1397.

Loomba, R., Abraham, M., Unalp, A., Wilson, L., Lavine, J., Doo, E., & Bass, N. M. (2012). Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. *Hepatology*, *56*(3), 943–951.

Loyd, C., Magrisso, I. J., Haas, M., Balusu, S., Krishna, R., Itoh, N., … Habegger, K. M. (2016). Fibroblast growth factor 21 is required for beneficial effects of exercise during chronic high-fat feeding. *Journal of Applied Physiology, 121*(3), 687-698.

Ludwig, J., Viggiano, T. R., McGill, D. B., & Ott, B. J. (1980). Nonalcoholic steatohepatitis: Mayo clinic experiences with a hitherto unnamed disease. *Mayo Clinic Proceedings*, *55*, 434–438.

Lydersen, S. (2015). Statistical review: frequently given comments. *Annals of the Rheumatic Diseases*, *74*(2), 323–325.

MacDougall, J. D., Hicks, A. L., MacDonald, J. R., McKelvie, R. S., Green, H. J., & Smith, K. M. (1998). Muscle performance and enzymatic adaptations to sprint interval training. *Journal of Applied Physiology*, *84*, 2138–2142.

Machado, M., Marques-Vidal, P., & Cortez-Pinto, H. (2006). Hepatic histology in obese patients undergoing bariatric surgery. *Journal of Hepatology*, *45*(4), 600–606.

MacInnis, M. J., & Gibala, M. J. (2016). Physiological adaptations to interval training and the role of exercise intensity. *The Journal of Physiology*, *9*, 2915–2930.

MacPherson, R. E. K., Hazell, T. J., Olver, T. D., Paterson, D. H., & Lemon, P. W. R. (2011). Run sprint interval training improves aerobic performance but not maximal cardiac output. *Medicine and Science in Sports and Exercise*, *43*(1), 115–122.

Maersk, M., Belza, A., Stødkilde-Jørgensen, H., Ringgaard, S., Chabanova, E., Thomsen, H., … Richelsen, B. (2012). Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study. *The American Journal of Clinical Nutrition*, *95*(2), 283–289.

Mahady, S. E., Webster, A. C., Walker, S., Sanyal, A., & George, J. (2011). The role of thiazolidinediones in non-alcoholic steatohepatitis - A systematic review and meta analysis. *Journal of Hepatology*, *55*(6), 1383–1390.

Malin, S., Del Rincon, J. P., Huang, H., & Kirwan, J. P. (2014). Exercise-induced lowering of fetuin-a may increase hepatic insulin sensitivity. *Medicine and Science in Sports and Exercise*, *46*(11), 2085–2090.

Malin, S., Haus, J., Solomon, T., Blaszczak, A., Kashyap, S., & Kirwan, J. (2013). Insulin sensitivity and metabolic flexibility following exercise training among different obese insulin-resistant phenotypes. *American Journal of Physiology. Endocrinology and Metabolism*, *305*(10), E1292-E1298.

Malin, S., Mulya, A., Fealy, C., Haus, J., Pagadala, M., Scelsi, A., … Kirwan, J. (2013). Fetuin-A is linked to improved glucose tolerance after short-term exercise training in nonalcoholic fatty liver disease. *Journal of Applied Physiology*, *115*(7), 988–994.

Mantovani, A., Byrne, C. D., Bonora, E., & Targher, G. (2018). Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: A meta-analysis. *Diabetes Care*, *41*(2), 372–382.

Maraki, M., & Sidossis, L. (2013). The latest on the effect of prior exercise on postprandial lipaemia. *Sports Medicine*, *43*(6), 463–481.

Marchesini, G., Day, C., Dufour, J.-F., Canbay, A., Nobili, V., Ratziu, V., … Mathus-Vliegen, L. (2016). EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol*, *64*, 1388–1402.

Marchesini, G., Petta, S., & Dalle Grave, R. (2016). Diet, weight loss, and liver health in NAFLD: Pathophysiology, evidence and practice. *Hepatology*, *63*, 2032–2043.

Markan, K. R., Naber, M. C., Ameka, M. K., Anderegg, M. D., Magelsdorf, D. J., Kliewer, S. A., … Potthoff, M. J. (2014). Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. *Diabetes*, *63*(12), 4057-4063.

Martinez, N., Kilpatrick, M. W., Salomon, K., Jung, M. E., & Little, J. P. (2015). Affective and enjoyment responses to high-intensity interval training in overweight-to-obese and insufficiently active adults. *Journal of Sport and Exercise Psychology*, *37*(2), 138–149.

Mashili, F. L., Austin, R. L., Deshmukh, A. S., Fritz, T., Caidahl, K., Bergdahl, K., … Krook, A. (2011). Direct effects of FGF21 on glucose uptake in human skeletal muscle: implications for type 2 diabetes and obesity. *Diabetes/metabolism Research and Reviews*, *27*, 286–297.

Mathews, S. T., Singh, G. P., Ranalletta, M., Cintron, V. J., Qiang, X., Goustin, A. S., … Grunberger, G. (2002). Improved insulin sensitivity and resistance to weight gain in mice null for the Ahsg gene. *Diabetes*, *51*(8), 2450–2458.

Matsuda, M., & DeFronzo, R. A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. *Diabetes Care*, *22*(9), 1462–1470.

Matsusue, K., Haluzik, M., Lambert, G., Yim, S. H., Gavrilova, O., Ward, J. M., … Gonzalez, F. J. (2003). Liver-specific disruption of PPARγ in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. *Journal of Clinical Investigation*, *111*(5), 737–747.

Matthews, D. R., Hosker, J. R., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, *28*(7), 412–419.

Mazzella, N., Ricciardi, L., Mazzotti, A., & Marchesini, G. (2014). The role of medications for the management of patients with NAFLD. *Clinics in Liver Disease*, *18*(1), 73–89.

McGarry, J., & Foster, D. (1980). Regulation of hepatic fatty acid oxidation and ketone body production. *Annual Review of Biochemistry*, *49*, 395–420.

McGown, C., Birerdinc, A., & Younossi, Z. M. (2014). Adipose tissue as an endocrine organ. *Clinics in Liver Disease*, *18*(1), 41–58.

McPherson, S., Hardy, T., Henderson, E., Burt, A. D., Day, C. P., & Anstee, Q. M. (2015). Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: Implications for prognosis and clinical management. *Journal of Hepatology*, *62*(5), 1148–1155.

Meex, R. C., Hoy, A. J., Morris, A., Brown, R. D., Lo, J. C. Y., Burke, M., … Watt, M. J. (2015). Fetuin B is a secreted hepatocyte factor linking steatosis to impaired glucose metabolism. *Cell Metabolism*, *22*(6), 1078–1089.

Meex, R. C. R., Schrauwen-Hinderling, V. B., Moonen-Kornips, E., Schaart, G., Mensink, M., Phielix, E., … Hesselink, M. K. C. (2010). Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. *Diabetes*, *59*(3), 572–579.

Meex, R. C. R., & Watt, M. J. (2017). Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. *Nature Reviews Endocrinology*, *13*(9), 509–520.

Melanson, E. L. (2017). The effect of exercise on non-exercise physical activity and sedentary behavior in adults. *Obesity Reviews*, *18*(2), 40–49.

Metcalfe, R. S., Babraj, J. A., Fawkner, S. G., & Vollaard, N. B. J. (2012). Towards the minimal amount of exercise for improving metabolic health: Beneficial effects of reduced-exertion high-intensity interval training. *European Journal of Applied Physiology*, *112*(7), 2767–2775.

Mifflin, M. D., St Jeor, S. T., Hill, L. A., Scott, B. J., Daugherty, S. A., & Koh, Y. O. (1990). A new predictive equation for resting energy expenditure in healthy individuals. *American Journal of Clinical Nutrition*, *51*(2), 241–247.

Miller, E. I., Pastor-Barriuso, R., Dalal, D., Riemersma, R., Appel, L., & Guallar, E. (2005). Meta-analysis: High-dosage vitamin E supplementation may increase all cause mortality. *Annals of Internal Medicine*, *142*, 37–46.

Misu, H., Ishikura, K., Kurita, S., Takeshita, Y., Ota, T., Saito, Y., … Takamura, T. (2012). Inverse correlation between serum levels of selenoprotein p and adiponectin in patients with type 2 diabetes. *PLoS ONE*, *7*(4), 1–7.

Misu, H., Takamura, T., Takayama, H., Hayashi, H., Matsuzawa-Nagata, N., Kurita, S., … Kaneko, S. (2010). A liver-derived secretory protein, selenoprotein P, causes insulin resistance. *Cell Metabolism*, *12*(5), 483–495.

Miyazaki-Anzai, S., Masuda, M., Levi, M., Keenan, A. L., & Miyazaki, M. (2014). Dual activation of the bile acid nuclear receptor FXR and G-Protein-Coupled receptor TGR5 protects mice against atherosclerosis. *PLoS ONE*, *9*(9), e108270.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Annals of Internal Medicine*, *151*(4), 264–269.

Montesi, L., Caselli, C., Centis, E., Nuccitelli, C., Moscatiello, S., Suppini, A., & Marchesini, G. (2014). Physical activity support or weight loss counseling for nonalcoholic fatty liver disease? *World Journal of Gastroenterology*, *20*(29), 10128–10136. h

Moore, M. C., Coate, K. C., Winnick, J. J., An, Z., & Cherrington, A. D. (2012). Regulation of hepatic glucose uptake and storage in vivo. *Advances in Nutrition*, *3*(2), 286–294.

Mori, K., Emoto, M., Araki, T., Yokoyama, H., Lee, E., Teramura, M., … Nishizawa, Y. (2008). Effects of pioglitazone on serum fetuin-A levels in patients with type 2 diabetes mellitus. *Metabolism: Clinical and Experimental*, *57*(9), 1248–1252.

Mraz, M., Bartlova, M., Lacinova, Z., Michalsky, D., Kasalicky, M., Haluzikova, D., … Haluzik, M. (2009). Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor-21 in patients with type 2 diabetes and obesity. *Clinical Endocrinology*, *71*(3), 369–375.

Mudaliar, S., Henry, R. R., Sanyal, A. J., Morrow, L., Marschall, H. U., Kipnes, M., … Shapiro, D. (2013). Efficacy and safety of the farnesoid x receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology*, *145*(3), 574–582.

Musso, G., Gambino, R., & Cassader, M. (2009). Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). *Progress in Lipid Research*, *48*(1), 1–26.

Musso, G., Gambino, R., Tabibian, J. H., Ekstedt, M., Kechagias, S., Hamaguchi, M., … Cassader, M. (2014). Association of non-alcoholic fatty liver disease with chronic kidney disease: A systematic review and meta-analysis. *PLoS Medicine*, *11*(7), e1001680.

Nagle, C. A., An, J., Shiota, M., Torres, T. P., Cline, G. W., Liu, Z. X., … Coleman, R. A. (2007). Hepatic overexpression of glycerol-sn-3-phosphate acyltransferase 1 in rats causes insulin resistance. *Journal of Biological Chemistry*, *282*(20), 14807–14815.

Nakai, R., Azuma, T., Kishimoto, T., Hirata, T., Takizawa, O., Hyon, S. H., & Tsutsumi, S. (2010). Development of a high-precision image-processing automatic measurement system for MRI visceral fat images acquired using a binomial RF-excitation pulse. *Magnetic Resonance Imaging*, *28*(4), 520–526.

NCD Risk Factor Collaboration. (2016). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *Lancet*, *387*(10026), 1377–1396.

Neschen, S., Morino, K., Hammond, L. E., Zhang, D., Liu, Z. X., Romanelli, A. J., … Shulman, G. I. (2005). Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 knockout mice. *Cell Metabolism*, *2*(1), 55–65.

Neuschwander-Tetri, B. A., Loomba, R., Sanyal, A. J., Lavine, J. E., Van Natta, M. L., Abdelmalek, M. F., … Doo, E. (2015). Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): A multicentre, randomised, placebo-controlled trial. *The Lancet*, *385*, 956–965.

Nielsen, S., Guo, Z., Johnson, C. M., Hensrud, D. D., & Jensen, M. D. (2004). Splanchnic lipolysis in human obesity. *The Journal of Clinical Investigation*, *113*(11), 1582–1588.

Obuchi, A., Adachi, H., Enomoto, M., Fukami, A., Kumagai, E., Nakamura, S., … Imaizumi, T. (2014). High plasma fetuin-A levels are associated with metabolic syndrome among males but not females in a Japanese general population. *Diabetes Research and Clinical Practice*, *106*(1), 128–135.

Oh, S., Shida, T., Sawai, A., Maruyama, T., Eguchi, K., Isobe, T., … Shoda, J. (2014). Acceleration training for managing nonalcoholic fatty liver disease: a pilot study. *Therapeutics and Clinical Risk Management*, *10*, 925–936.

Oh, S., Shida, T., Yamagishi, K., Tanaka, K., So, R., Tsujimoto, T., & Shoda, J. (2015). Moderate to vigorous physical activity volume is an important factor for managing nonalcoholic fatty liver disease: A retrospective study. *Hepatology*, *61*, 1205–1215.

Oh, S., So, R., Shida, T., Matsuo, T., Kim, B., Akiyama, K., … Shoda, J. (2017). High-intensity aerobic exercise improves both hepatic fat content and stiffness in sedentary obese men with nonalcoholic fatty liver disease. *Scientific Reports*, *7*(2), 1–12.

Okumura, A., Unoki-Kubota, H., Matsushita, Y., Shiga, T., Moriyoshi, Y., Yamagoe, S., & Y, K. (2013). Increased serum leukocyte cell-derived chemotaxin 2 (LECT2) levels in obesity and fatty liver. *BioScience Trends*, *7*(6), 276–283.

Orci, L. A., Gariani, K., Oldani, G., Delaune, V., Morel, P., & Toso, C. (2016). Exercise-based interventions for nonalcoholic fatty liver disease: A meta-analysis and meta-regression. *Clinical Gastroenterology and Hepatology*, *14*(10), 1398–1411.

Ou, H.-Y., Wu, H.-T., Hung, H.-C., Yang, Y.-C., Wu, J.-S., & Chang, C.-J. (2012). Endoplasmic reticulum stress induces the expression of fetuin-A to develop insulin resistance. *Endocrinology*, *153*(7), 2974–2984.

Ou, H.-Y., Yang, Y.-C., Wu, H.-T., Wu, J.-S., Lu, F.-H., & Chang, C.-J. (2012). Increased fetuin-A concentrations in impaired glucose tolerance with or without nonalcoholic fatty liver disease, but not impaired glucose tolerance. *Journal of Clinical Endocrinology & Metabolism*, *97*(12), 4717–4723.

Özcan, U., Cao, Q., Yilmaz, E., Lee, A.-H., Iwakoshi, N. N., Özdelen, E., … Hotamisligil, G. S. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science*, *306*, 457–461.

Özcan, U., Yilmaz, E., Özcan, L., Furuhashi, M., Vaillancourt, E., Smith, R. O., … Hotamisligil, G. S. (2006). Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science*, *313*, 1137–1140.

Pal, D., Dasgupta, S., Kundu, R., Maitra, S., Das, G., Mukhopadhyay, S., … Bhattacharya, S. (2012). Fetuin-A as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nature Medicine*, *18*(8), 1279–1288.

Pardina, E., Baena-Fustegueras, J. A., Llamas, R., Catalán, R., Galard, R., Lecube, A., … Peinado-Onsurbe, J. (2009). Lipoprotein lipase expression in livers of morbidly obese patients could be responsible for liver steatosis. *Obesity Surgery*, *19*(5), 608–616.

Pardina, E., Ferrer, R., Rossell, J., Ricart-Jane, D., Mendez-Lara, K. A., Baena-Fustegueras, J. A., … Peinado-Onsurbe, J. (2017). Hepatic CD36 downregulation parallels steatosis improvement in morbidly obese undergoing bariatric surgery. *International Journal of Obesity*, (17), 1388–1393.

Patel, N. S., Doycheva, I., Peterson, M. R., Hooker, J., Kisselva, T., Schnabl, B., … Loomba, R. (2015). Effect of weight loss on magnetic resonance imaging estimation of liver fat and volume in patients with nonalcoholic steatohepatitis. *Clinical Gastroenterology and Hepatology*, *13*(3), 561–568.

Patterson, E., Wall, R., Fitzgerald, G., Ross, R., & Stanton, C. (2012). Health implications of high dietary omega-6 polyunsaturated fatty acids. *Journal of Nutrition and Metabolism*, 1–16.

Perry, R. J., Samuel, V. T., Petersen, K. F., & Shulman, G. I. (2014). The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature*, *510*, 84–91.

Petersen, K. F., Dufour, S., Befroy, D., Lehrke, M., Hendler, R. E., & Shulman, G. I. (2005). Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes*, *54*(3), 603–608.

Petersen, K. F., Dufour, S., Savage, D. B., Bilz, S., Solomon, G., Yonemitsu, S., … Shulman, G. I. (2007). The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proceedings of the National Academy of Sciences*, *104*(31), 12587–12594.

Petersen, M. C., Vatner, D. F., & Shulman, G. I. (2017). Regulation of hepatic glucose metabolism in health and disease. *Nature Reviews Endocrinology*, *13*(10), 572–587.

Pettinelli, P., del Pozo, T., Araya, J., Rodrigo, R., Araya, A. V., Smok, G., … Videla, L. A. (2009). Enhancement in liver SREBP-1c/PPAR-α ratio and steatosis in obese patients: Correlations with insulin resistance and n-3 long-chain polyunsaturated fatty acid depletion. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1792*(11), 1080–1086.

Peverill, W., Powell, L. W., & Skoien, R. (2014). Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *International Journal of Molecular Sciences*, *15*(5), 8591–8638.

Phillips, D. J., & de Kretser, D. M. (1998). Follistatin: a multifunctional regulatory protein. *Frontiers in Neuroendocrinology*, *19*(4), 287–322.

Polak, J., Moro, C., Klimcakova, E., Hejnova, J., Majercik, M., Viguerie, N., … Berlan, M. (2005). Dynamic strength training improves insulin sensitivity and functional balance between adrenergic alpha 2A and beta pathways in subcutaneous adipose tissue of obese subjects. *Diabetologia*, *48*(12), 2631–2640.

Pontzer, H., Durazo-Arvizu, R., Dugas, L. R., Plange-Rhule, J., Bovet, P., Forrester, T. E., … Luke, A. (2016). Constrained total energy expenditure and metabolic adaptation to physical activity in adult humans. *Current Biology*, *26*(3), 410–417.

Portillo Sanchez, P., Bril, F., Maximos, M., Lomonaco, R., Biernacki, D., Orsak, B., … Cusi, K. (2015). High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *The Journal of Clinical Endocrinology and Metabolism*, *100*(5), 1–9.

Potthoff, M. J., Kliewer, S. A., & Mangelsdorf, D. J. (2012). Endocrine fibroblast growth factors 15/19 and 21: From feast to famine. *Genes & Development*, *26*(4), 312–324.

Promrat, K., Kleiner, D. E., Niemeier, H. M., Jackvony, E., Kearns, M., Wands, J. R., … Wing, R. R. (2010). Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology*, *51*(1), 121–129.

Pugh, C. J. A., Sprung, V. S., Jones, H., Richardson, P., Shojaee-Moradie, F., Umpleby, A. M., … Cuthbertson, D. J. (2016). Exercise-induced improvements in liver fat and endothelial function are not sustained 12 months following cessation of exercise supervision in non-alcoholic fatty liver disease (NAFLD). *International Journal of Obesity*, *40*(12), 1927–1930.

Pugh, C., Sprung, V., Kemp, G., Richardson, P., Shojaee-Moradie, F., Umpleby, A., … Cuthbertson, D. (2014). Exercise training reverses endothelial dysfunction in nonalcoholic fatty liver disease. *American Journal of Physiology: Heart and Circulatory Physiology*, *307*(9), H1298–H1306.

Puri, P., Mirshahi, F., Cheung, O., Natarajan, R., Maher, J. W., Kellum, J. M., & Sanyal, A. J. (2008). Activation and dysregulation of the unfolded protein response in nonalcoholic fatty liver disease. *Gastroenterology*, *134*(2), 568–576.

Rabøl, R., Petersen, K. F., Dufour, S., Flannery, C., & Shulman, G. I. (2011). Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(33), 13705–13709.

Raddatz, K., Turner, N., Frangioudakis, G., Liao, B. M., Pedersen, D. J., Cantley, J., … Schmitz-Peiffer, C. (2011). Time-dependent effects of *Prkce* deletion on glucose homeostasis and hepatic lipid metabolism on dietary lipid oversupply in mice. *Diabetologia*, *54*(6), 1447–1456.

Ratziu, V., Goodman, Z., & Sanyal, A. (2015). Current efforts and trends in the treatment of NASH. *Journal of Hepatology*, *62*(S1), S65–S75.

Rector, R. S., Thyfault, J. P., Morris, R. T., Laye, M. J., Borengasser, S. J., Booth, F. W., & Ibdah, J. A. (2008). Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in otsuka long-evans tokushima fatty rats. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *294*(3), G619-26.

Rector, R. S., Uptergrove, G. M., Morris, E. M., Borengasser, S. J., Laughlin, M. H., Booth, F. W., … Ibdah, J. A. (2011). Daily exercise vs. caloric restriction for prevention of nonalcoholic fatty liver disease in the OLETF rat model. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *300*(5), G874–G883.

Reinehr, T., & Roth, C. L. (2008). Fetuin-A and its relation to metabolic syndrome and fatty liver disease in obese children before and after weight loss. *The Journal of Clinical Endocrinology and Metabolism*, *93*(11), 4479–4485.

Reitman, M. L. (2013). FGF21 mimetic shows therapeutic promise. *Cell Metabolism*, *18*(3), 307–309.

Richards, J. C., Johnson, T. K., Kuzma, J. N., Lonac, M. C., Schweder, M. M., Voyles, W. F., & Bell, C. (2010). Short-term sprint interval training increases insulin sensitivity in healthy adults but does not affect the thermogenic response to beta-adrenergic stimulation. *The Journal of Physiology*, *588*(15), 2961–2972.

Riebe, D., Franklin, B. A., Thompson, P. D., Garber, C. E., Whitfield, G. P., Magal, M., & Pescatello, L. S. (2015). Updating ACSM's recommendations for exercise preparticipation health screening. *Medicine and Science in Sports and Exercise*, *47*(11), 2473–2479.

Rinella, M. E. (2015). Nonalcoholic fatty liver disease: A systematic review. *Journal of the American Medical Association*, *313*(22), 2263-2273.

Rinella, M. E., & Sanyal, A. J. (2016). Management of NAFLD: a stage-based approach. *Nature Reviews Gastroenterology and Hepatology*, *13*(4), 196–205.

Rizza, R. A. (2010). Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: Implications for therapy. *Diabetes*, *59*(11), 2697–2707.

Rosqvist, F., Iggman, D., Kullberg, J., Cedernaes, J., Johansson, H. E., Larsson, A., … Risérus, U. (2014). Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes*, *63*(7), 2356–2368.

Ross, R., Freeman, J., & Janssen, I. (2000). Exercise alone is an effective strategy for reducing obesity and related comorbidities. *Exercise and Sport Sciences Reviews*, *28*(4), 165–170.

Ryan, M. C., Itsiopoulos, C., Thodis, T., Ward, G., Trost, N., Hofferberth, S., … Wilson, A. M. (2013). The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *Journal of Hepatology*, *59*(1), 138–143.

Sadur, C. N., Yost, T. J., & Eckel, R. H. (1984). Fat feeding decreases insulin responsiveness of adipose tissue lipoprotein lipase. *Metabolism*, *33*(11), 1043–1047.

Saito, Y., & Takahashi, K. (2002). Characterization of selenoprotein P as a selenium supply protein. *European Journal of Biochemistry*, *269*(22), 5746–5751.

Saito, Y., Watanabe, Y., Saito, E., Honjoh, T., & Takahashi, K. (2001). Production and application of monoclonal antibodies to human selenoprotein P. *Journal of Health Science*, *47*(4), 346–352.

Salmon, J., Owen, N., Crawford, D., Bauman, A., & Sallis, J. F. (2003). Physical activity and sedentary behavior: A population-based study of barriers, enjoyment, and preference. *Health Psychology*, *22*(2), 178–188.

Samocha-Bonet, D., Tam, C. S., Campbell, L. V., & Heilbronn, L. K. (2014). Raised circulating fetuin-A after 28-day overfeeding in healthy humans. *Diabetes Care*, *37*(1), 15–16.

Samuel, V. T., Liu, Z.-X., Wang, A., Beddow, S. A., Geisler, J. G., Kahn, M., … Shulman, G. I. (2007). Inhibition of protein kinase Cε prevents hepatic insulin resistance in nonalcoholic fatty liver disease. *Journal of Clinical Investigation*, *117*(3), 739–745.

Samuel, V. T., Liu, Z. X., Qu, X., Elder, B. D., Bilz, S., Befroy, D., … Shulman, G. I. (2004). Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *Journal of Biological Chemistry*, *279*(31), 32345–32353.

Samuel, V. T., Petersen, K. F., & Shulman, G. I. (2010). Lipid-induced insulin resistance : unravelling the mechanism. *Lancet*, *375*, 2267–2277.

Samuel, V. T., & Shulman, G. I. (2012). Mechanisms for insulin resistance: Common threads and missing links. *Cell*, *148*(5), 852–871.

Sanyal, A. J., Abdelmalek, M. F., Suzuki, A., Cummings, O. W., & Chojkier, M. (2014). No significant effects of ethyl-eicosapentanoic acid on histologic features of nonalcoholic steatohepatitis in a phase 2 triaL. *Gastroenterology*, *147*(2), 377–384.

Sanyal, A. J., Campbell-Sargent, C., Mirshahi, F., Rizzo, W. B., Contos, M. J., Sterling, R. K., … Clore, J. N. (2001). Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities. *Gastroenterology*, *120*(5), 1183–1192.

Sanyal, A. J., Chalasani, N., Kowdley, K. V, McCullough, A. J., Diehl, A. M., Bass, N. M., … Robuck, P. R. (2010). Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *New England Journal of Medicine*, *362*, 1675–1685.

Sargeant, J. A., Aithal, G. P., Takamura, T., Misu, H., Takayama, H., Douglas, J., … King, J. A. (2018). The influence of adiposity and acute exercise on circulating hepatokines in normal weight and overweight/obese men. *Applied Physiology Nutrition and Metabolism*, *43*, 482–490.

Sargeant, J. A., Bawden, S., Aithal, G. P., Simpson, E. J., Macdonald, I. A., Turner, M. C., … King, J. A. (2018). Effects of sprint interval training on ectopic lipids and tissue-specific insulin sensitivity in men with non-alcoholic fatty liver disease. *Eur J Appl Physiol*, *118*(4), 817–828.

Sargeant, J. A., Gray, L. J., Bodicoat, D. H., Willis, S. A., Stensel, D. J., Nimmo, M. A., … King, J. A. (2018). The effect of exercise training on intrahepatic triglyceride and heaptic insulin sensitivity: A systematic review and meta-analysis. *Obesity Reviews*, *19*, 1446-1459.

Savage, D. B., Tan, G. D., Acerini, C. L., Jebb, S. A., Agostini, M., Gurnell, M., … O'Rahilly, S. (2003). Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-γ. *Diabetes*, *52*(4), 910–917.

Scalzo, R. L., Peltonen, G. L., Giordano, G. R., Binns, S. E., Klochak, A. L., Paris, H. L. R., … Bell, C. (2014). Regulators of human white adipose browning: Evidence for sympathetic control and sexual dimorphic responses to sprint interval training. *PLoS ONE*, *9*(3), e90696.

Schauer, P. R., Bhatt, D. L., Kirwan, J. P., Wolski, K., Aminian, A., Brethauer, S. A., … Kashyap, S. R. (2017). Bariatric surgery versus intensive medical therapy for diabetes — 5-year outcomes. *New England Journal of Medicine*, *376*(7), 641–651.

Schenk, S., Saberi, M., & Olefsky, J. M. (2008). Insulin sensitivity: Modulation by nutrients and inflammation. *Journal of Clinical Investigation*, *118*(9), 2992–3002.

Schubert, M. M., Palumbo, E., Seay, R. F., Spain, K. K., & Clarke, H. E. (2017). Energy compensation after sprint- and high-intensity interval training. *PLoS One, 12*(12), e0189590.

Schultes, B., Frick, J., Ernst, B., Stefan, N., & Fritsche, A. (2010). The effect of 6-weeks of aerobic exercise training on serum fetuin-A levels in non-diabetic obese women. *Experimental and Clinical Endocrinology & Diabetes*, *118*(10), 754–756.

Schupp, M., & Lazar, M. A. (2010). Endogenous ligands for nuclear receptors: Digging deeper. *Journal of Biological Chemistry*, *285*(52), 40409–40415.

Schwartz, M. W., Seeley, R. J., Zeltser, L. M., Drewnowski, A., Ravussin, E., Redman, L. M., & Leibel, R. L. (2017). Obesity pathogenesis: An endocrine society scientific statement. *Endocrine Reviews*, *38*(4), 267–296.

Schwarz, J. M., Linfoot, P., Dare, D., & Aghajanian, K. (2003). Hepatic de novo lipogenesis in normoinsulinemic and hyperinsulinemic subjects consuming high-fat, low-carbohydrate and low-fat, high-carbohydrate isoenergetic diets. *American Journal of Clinical Nutrition*, *77*(1), 43–50.

Segal, K., Edano, A., Abalos, A., Albu, J., Blando, L., Tomas, M., & Pi-Sunyer, F. (1991). Effect of exercise training on insulin sensitivity and glucose metabolism in lean, obese, and diabetic men. *Journal of Applied Physiology*, *71*(6), 2402–2411.

Seppälä-Lindroos, A., Vehkavaara, S., Häkkinen, A. M., Goto, T., Westerbacka, J., Sovijärvi, A., … Yki-Järvinen, H. (2002). Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *Journal of Clinical Endocrinology and Metabolism*, *87*(7), 3023–3028.

Sevastianova, K., Santos, A., Kotronen, A., Hakkarainen, A., Makkonen, J., Silander, K., … Yki-Jaŕvinen, H. (2012). Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans. *American Journal of Clinical Nutrition*, *96*(4), 727–734.

Shen, W., Wang, Z., Punyanita, M., Lei, J., Sinav, A., Kral, J. G., … Heymsfield, S. B. (2003). Adipose tissue quantification by imaging methods: A proposed classification. *Obesity Research*, *11*(1), 5–16.

Shepherd, S. O., Cocks, M., Tipton, K. D., Ranasinghe, A. M., Barker, T. A., Burniston, J. G., … Shaw, C. S. (2013). Sprint interval and traditional endurance training increase net intramuscular triglyceride breakdown and expression of perilipin 2 and 5. *The Journal of Physiology*, *591*(Pt 3), 657–675.

Sherk, V. D., Chrisman, C., Smith, J., Young, K. C., Singh, H., Bemben, M. G., & Bemben, D. A. (2013). Acute bone marker responses to whole-body vibration and resistance exercise in young women. *Journal of Clinical Densitometry*, *16*(1), 104–109.

Shoelson, S. E., Herrero, L., & Naaz, A. (2007). Obesity, inflammation, and insulin resistance. *Gastroenterology*, *132*(6), 2169–2180.

Shojaee-Moradie, F., Baynes, K. C. R., Pentecost, C., Bell, J. D., Thomas, E. L., Jackson, N. C., … Umpleby, A M. (2007). Exercise training reduces fatty acid availability and improves the insulin sensitivity of glucose metabolism. *Diabetologia*, *50*(2), 404–413.

Shojaee-Moradie, F., Cuthbertson, D. J., Barrett, M., Jackson, N. C., Herring, R., Thomas, E. L., … Umpleby, A. M. (2016). Exercise training reduces liver fat and increases rates of VLDL clearance but not VLDL production in NAFLD. *Journal of Clinical Endocrinology and Metabolism*, *101*(11), 4219–4228.

Shulman, G. I. (2014). Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *The New England Journal of Medicine*, *371*(12), 1131–1141.

Siddiq, A., Lepretre, F., Hercberg, S., Froguel, P., & Gibson, F. (2005). A synonymous coding polymorphism in the alpha2-heremans-schmid glycoprotein gene is associated with type 2 diabetes in French caucasians. *Diabetes*, *54*, 2477–2481.

Singh, S., Allen, A. M., Wang, Z., Prokop, L. J., Murad, M. H., & Loomba, R. (2015). Fibrosis progression in nonalcoholic fatty liver versus nonalcoholic steatohepatitis: A systematic review and meta- analysis of paired-biopsy studies. *Clinical Gastroenterology and Hepatology*, *13*(4), 643–654.

Sinn, D. H., Kang, D., Jang, H. R., Gu, S., Cho, S. J., Paik, S. W., … Gwak, G. (2017). Development of chronic kidney disease in patients with non-alcoholic fatty liver disease: A cohort study. *Journal of Hepatology*, *67*, 1274–1280.

Slentz, C. A., Bateman, L. A., Willis, L. H., Shields, A. T., Tanner, C. J., Piner, L. W., … Kraus, W. E. (2011). Effects of aerobic vs. resistance training on visceral and liver fat stores, liver enzymes, and insulin resistance by HOMA in overweight adults from STRRIDE AT/RT. *American Journal of Physiology. Endocrinology and Metabolism*, *301*(5), E1033-E1039.

Slusher, A. L., Whitehurst, M., Zoeller, R. F., Mock, J. T., Maharaj, M., & Huang, C.-J. (2015). Attenuated fibroblast growth factor 21 response to acute aerobic exercise in obese individuals. *Nutrition, Metabolism, and Cardiovascular Diseases*, *25*(9), 839–845.

Smart, N. A., King, N., McFarlane, J. R., Graham, P. L., & Dieberg, G. (2016). Effect of exercise training on liver function in adults who are overweight or exhibit fatty liver disease: a systematic review and meta-analysis. *British Journal of Sports Medicine*, 1–11.

Sobrecases, H., Lê, K. A., Bortolotti, M., Schneiter, P., Ith, M., Kreis, R., … Tappy, L. (2010). Effects of short-term overfeeding with fructose, fat and fructose plus fat on plasma and hepatic lipids in healthy men. *Diabetes and Metabolism*, *36*(3), 244–246.

Söderberg, C., Stål, P., Askling, J., Glaumann, H., Lindberg, G., Marmur, J., & Hultcrantz, R. (2010). Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology*, *51*(2), 595–602.

Solomon, T. P. J., Haus, J. M., Marchetti, C. M., Stanley, W. C., & Kirwan, J. P. (2009). Effects of exercise training and diet on lipid kinetics during free fatty acid-induced insulin resistance in older obese humans with impaired glucose tolerance. *American Journal of Physiology. Endocrinology and Metabolism*, *297*(2), E552–E559.

Song, A., Xu, M., Bi, Y., Xu, Y., Huang, Y., Li, M., … Ning, G. (2011). Serum fetuin-A associates with type 2 diabetes and insulin resistance in Chinese adults. *PLoS ONE*, *6*(4).

Speckmann, B., Walter, P. L., Alili, L., Reinehr, R., Sies, H., Klotz, L. O., & Steinbrenner, H. (2008). Selenoprotein P expression is controlled through interaction of the coactivator PGC-1α with FoxO1a and hepatocyte nuclear factor 42α transcription factors. *Hepatology*, *48*(6), 1998–2006.

Srinivas, P., Wagner, A., Reddy, L., Deutch, D., Leon, M., Goustin, A., & Grunberger, G. (1993). Serum alpha2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. *Molecular Endocrinology*, *7*(11), 1445–1455.

Stefan, N., & Häring, H.-U. (2011). The metabolically benign and malignant fatty liver. *Diabetes*, *60*(8), 2011–2017.

Stefan, N., & Häring, H.-U. (2013). The role of hepatokines in metabolism. *Nature Reviews. Endocrinology*, *9*(3), 144–152.

Stefan, N., Hennige, A. M., Staiger, H., Machann, J., Schick, F., Krober, S. M., … Haring, H.-U. (2006). α2-Heremans-schmid glycoprotein/ fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. *Diabetes Care*, *29*(4), 853–857.

Stefan, N., Sun, Q., Fritsche, A., Machann, J., Schick, F., Gerst, F., … Schulze, M. B. (2014). Impact of the adipokine adiponectin and the hepatokine fetuin-A on the development of type 2 diabetes: Prospective cohort- and cross-sectional phenotyping studies. *PloS One*, *9*(3), e92238.

Stephens, N. A., & Sparks, L. M. (2015). Resistance to the beneficial effects of exercise in type 2 diabetes: Are some individuals programmed to fail? *The Journal of Clinical Endocrinology and Metabolism*, *100*(1), 43–52.

Stephenson, M. C., Leverton, E., Khoo, E. Y. H., Poucher, S. M., Johansson, L., Lockton, J. A., … Macdonald, I. A. (2013). Variability in fasting lipid and glycogen contents in hepatic and skeletal muscle tissue in subjects with and without type 2 diabetes: A 1H and 13C MRS study. *NMR in Biomedicine*, *26*(11), 1518–1526.

Strasser, B., Pesta, D., Strasser, B., & Pesta, D. (2013). Resistance training for diabetes prevention and therapy: Experimental findings and molecular mechanisms. *BioMed Research International*, *2013*, 1–8.

Sullivan, S., Kirk, E. P., Mittendorfer, B., Patterson, B. W., & Klein, S. (2012). Randomized trial of exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease. *Hepatology*, *55*(6), 1738–1745.

Sung, K.-C., Wild, S. H., & Byrne, C. D. (2013). Resolution of fatty liver and risk of incident diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *98*(9), 3637–3643.

Sunny, N. E., Parks, E. J., Browning, J. D., & Burgess, S. C. (2011). Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metabolism*, *14*(6), 804–810.

Sylow, L., Kleinert, M., Richter, E. A., & Jensen, T. E. (2017). Exercise-stimulated glucose uptake - regulation and implications for glycaemic control. *Nature Reviews Endocrinology*, *13*(3), 133–148.

Szczepaniak, L. S., Nurenberg, P., Leonard, D., Browning, J. D., Reingold, J. S., Grundy, S., … Dobbins, R. L. (2005). Magnetic resonance spectroscopy to measure hepatic triglyceride content: Prevalence of hepatic steatosis in the general population. *American Journal of Physiology. Endocrinology and Metabolism*, *288*(2), E462-E468.

Szendroedi, J., & Roden, M. (2009). Ectopic lipids and organ function. *Current Opinion in Lipidology*, *20*(1), 50–56.

Takamura, T., Misu, H., & Kaneko, S. (2016). Ectopic fat accumulation in the liver and glucose homeostasis. In M. Inaba (Ed.), *Musculoskeletal Disease Associated with Diabetes Mellitus* (pp. 185–199). Springer Japan.

Takata, H., Ikeda, Y., Suehiro, T., Ishibashi, A., Inoue, M., Kumon, Y., & Terada, Y. (2009). High glucose induces transactivation of the gene through the ERK1/2 signaling pathway 2-HS glycoprotein. *Journal of Atherosclerosis and Thrombosis*, *16*(4), 448–456.

Tan, C. Y., & Vidal-Puig, A. (2008). Adipose tissue expandability: The metabolic problems of obesity may arise from the inability to become more obese. *Biochemical Society Transactions*, *36*(5), 935–940.

Tanaka, M., Saito, Y., Misu, H., Kato, S., Kita, Y., Takeshita, Y., … Matsuyama, N. (2016). Development of a sol particle homogeneous immunoassay for measuring full-length selenoprotein P in human serum. *Journal of Clinical Laboratory Analysis*, *30*(2), 114–122.

Taniguchi, H., Tanisawa, K., Sun, X., Cao, Z. B., Oshima, S., Ise, R., … Higuchi, M. (2014). Cardiorespiratory fitness and visceral fat are key determinants of serum fibroblast growth factor 21 concentration in Japanese men. *Journal of Clinical Endocrinology and Metabolism*, *99*(10), E1877–E1884.

Taniguchi, H., Tanisawa, K., Sun, X., Kubo, T., & Higuchi, M. (2016). Endurance exercise reduces hepatic fat content and serum fibroblast growth factor 21 levels in elderly men. *The Journal of Clinical Endocrinology and Metabolism*, *101*(1), 191–198.

Targher, G., Byrne, C. D., Lonardo, A., Zoppini, G., & Barbui, C. (2016). Nonalcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis of observational studies. *Journal of Hepatology*, *65*(3), 589–600.

Targher, G., Day, C. P., & Bonora, E. (2010). Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *The New England Journal of Medicine*, *363*, 1341–1350.

Taylor, R. (2008). Pathogenesis of type 2 diabetes: Tracing the reverse route from cure to cause. *Diabetologia*, *51*(10), 1781–1789.

Taylor, J., Keating, S. E., Holland, D. J., Coombes, J. S., & Leveritt, M. D. (2018). The chronic effect of interval training on energy intake: a systematic review and meta-analysis. *Journal of Obesity, 2018*, 1-13.

Tchkonia, T., Thomou, T., Zhu, Y., Karagiannides, I., Pothoulakis, C., Jensen, M. D., & Kirkland, J. L. (2013). Mechanisms and metabolic implications of regional differences among fat depots. *Cell Metabolism*, *17*(5), 644–656.

Teli, M., James, O. F. W., Burt, A. D., Bennett, M. K., & Day, C. P. (1995). The natural history of nonalcoholic fatty liver : A follow-up study. *Hepatology*, *22*, 1714–1719.

Than, N. N., & Newsome, P. N. (2015). A concise review of non-alcoholic fatty liver disease. *Atherosclerosis*, *239*(1), 192–202.

The Global BMI Mortality Collaboration. (2016). Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. *The Lancet*, *388*, 776–786.

Thomas, E. L., Parkinson, J. R., Frost, G. S., Goldstone, A. P., Doré, C. J., McCarthy, J. P., … Bell, J. D. (2012). The missing risk: MRI and MRS phenotyping of abdominal adiposity and ectopic fat. *Obesity*, *20*(1), 76–87.

Thompson, P. D., Franklin, B. A., Balady, G. J., Blair, S. N., Corrado, D., Estes, N. A. M., … Costa, F. (2007). Exercise and acute cardiovascular events: Placing the risks into perspective. A scientific statement from the American Heart Association council on nutrition, physical activity, and metabolism and the council on clinical cardiology. *Circulation*, *115*(17), 2358–2368.

Tiikkainen, M., Tamminen, M., Häkkinen, A.-M., Bergholm, R., Vehkavaara, S., Halavaara, J., … Yki-Järvinen, H. (2002). Liver-fat accumulation and insulin resistance in obese women with previous gestational diabetes. *Obesity Research*, *10*(9), 859–867.

Tilg, H., & Moschen, A. R. (2010). Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. *Hepatology*, *52*(5), 1836–1846.

Tjønna, A. E., Lee, S. J., Rognmo, Ø., Stølen, T. O., Bye, A., Haram, P. M., … Wisløff, U. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: A pilot study. *Circulation*, *118*(4), 346–354.

Trepanowski, J. F., Mey, J., & Varady, K. A. (2014). Fetuin-A: A novel link between obesity and related complications. *International Journal of Obesity, 39*(5), 734–741.

Troiano, R. P., Berrigan, D., Dodd, K. W., Mâsse, L. C., Tilert, T., Mcdowell, M., … Mcdowell, M. (2008). Physical activity in the United States measured by accelerometer. *Medicine and Science in Sports and Exercise*, *40*(1), 181–188.

Trost, S. G., Owen, N., Bauman, A. E., Sallis, J. F., & Brown, W. (2002). Correlates of adults' participation in physical activity: review and update. *Medicine and Science in Sports and Exercise*, *34*(12), 1996–2001.

Tsekouras, Y. E., Magkos, F., Kellas, Y., Basioukas, K. N., Kavouras, S. A, & Sidossis, L. S. (2008). High-intensity interval aerobic training reduces hepatic very low-density lipoprotein-triglyceride secretion rate in men. *American Journal of Physiology. Endocrinology and Metabolism*, *295*(4), E851–E858.

Turner, N., Kowalski, G. M., Leslie, S. J., Risis, S., Yang, C., Lee-Young, R. S., … Bruce, C. R. (2013). Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia*, *56*(7), 1638–1648.

Ueland, T., Aukrust, P., Aakhus, S., Smith, C., Endresen, K., Birkeland, K. I., … Johansen, O. E. (2012). Activin A and cardiovascular disease in type 2 diabetes mellitus. *Diabetes and Vascular Disease Research*, *9*(3), 234–237.

Umpierre, D., Ribeiro, P. A., Kramer, C. K., Leitao, C. B., Zucatti, A. T., Azevedo, M. J., … Schaan, B. D. (2011). Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: A systematic review and meta-analysis. *The Journal of the American Medical Association*, *305*(17), 1790–1799.

Van der Heijden, G.-J., Wang, Z., Chu, Z., Sauer, P., Haymond, M., Rodriguez, L., & Sunehag, A. (2010). A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. *Obesity*, *18*(2), 384–390.

Van der Heijden, G.-J., Wang, Z., Chu, Z., Toffolo, G., Manesso, E., Sauer, P., & Sunehag, A. (2010). Strength exercise improves muscle mass and hepatic insulin sensitivity in obese youth. *Medicine and Science in Sports and Exercise*, *42*(11), 1973–1980.

Van der Heijden, G., Toffolo, G., Manesso, E., Sauer, P., & Sunehag, A. (2009). Aerobic exercise increases peripheral and hepatic insulin sensitivity in sedentary adolescents. *Journal of Clinical Endocrinology and Metabolism*, *94*(11), 4292–4299.

Van Der Klaauw, A. A., & Farooqi, I. S. (2015). The hunger genes: Pathways to obesity. *Cell*, *161*(1), 119–132.

Van Herpen, N. A., Schrauwen-Hinderling, V. B., Schaart, G., Mensink, R. P., & Schrauwen, P. (2011). Three weeks on a high-fat diet increases intrahepatic lipid accumulation and decreases metabolic flexibility in healthy overweight men. *Journal of Clinical Endocrinology and Metabolism*, *96*(4), 691–695.

Van Wagner, L. B., Koppe, S. W., Brunt, E. M., Gottstein, J., Gardikiotes, K., Green, R. M., & Rinella, M. E. (2011). Pentoxifylline for the treatment of non-alcoholic steatohepatitis: a randomized controlled trial. *Annals of Hepatology*, *10*, 277–286.

Vella, A., & Rizza, R. A. (2009). Application of isotopic techniques using constant specific activity or enrichment to the study of carbohydrate metabolism. *Diabetes*, *58*(10), 2168–2174.

Vernon, G., Baranova, A., & Younossi, Z. M. (2011). Systematic review: The epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Alimentary Pharmacology & Therapeutics*, *34*(3), 274–285.

Vilar-Gomez, E., Martinez-Perez, Y., Calzadilla-Bertot, L., Torres-Gonzalez, A., Gra-Oramas, B., Gonzalez-Fabian, L., … Romero-Gomez, M. (2015). Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology*, *149*(2), 367–378.

Villareal, D. T., Aguirre, L., Gurney, A. B., Waters, D. L., Sinacore, D. R., Colombo, E., … Qualls, C. (2017). Aerobic or resistance exercise, or both, in dieting obese older adults. *The New England Journal of Medicine*, *376*, 1943–1955.

Villareal, D. T., Chode, S., Parimi, N., Sinacore, D. R., Hilton, T., Armamento-Villareal, R., … Shan, K. (2011). Weight loss, exercise, or both and physical function in obese older adults. *The New England Journal of Medicine*, *364*, 1218–1229.

Vollaard, N. B., Metcalfe, R. S., & Williams, S. (2017). Effect of number of sprints in a SIT session on change in $\dot{V}$O2max: A meta-analysis. *Medicine and Science in Sports and Exercise, 49*(6), 1147-1156.

Von Loeffelholz, C., Horn, P., Birkenfeld, A. L., Claus, R. A., Metzing, B. U., Döcke, S., … Sponholz, C. (2016). Fetuin A is a predictor of liver fat in preoperative patients with nonalcoholic fatty liver disease. *Journal of Investigative Surgery*, *29*(5), 266–274.

Wang, Y. C., McPherson, K., Marsh, T., Gortmaker, S. L., & Brown, M. (2011). Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet*, *378*, 815–825.

Wanless, I. R., & Lentz, J. S. (1990). Fatty liver hepatitis (steatohepatitis) and obesity : An autopsy study with analysis of risk factors. *Hepatology*, *12*, 1106–1110.

Washburn, R. A., Szabo, A. N., Lambourne, K., Willis, E. A., Ptomey, L. T., Honas, J. J., … Donnelly, J. E. (2014). Does the method of weight loss effect long-term changes in weight, body composition or chronic disease risk factors in overweight or obese adults? A systematic review. *PLoS ONE*, *9*(10), e109849.

Watt, M. J. (2009). Storing up trouble: Does accumulation of intramyocellular triglyceride protect skeletal muscle from insulin resistance? *Clinical and Experimental Pharmacology and Physiology*, *36*(1), 5–11.

Weisberg, S., McCann, D., Desai, M., Rosenbaum, M., Leibel, R., & Ferrante Jr, A. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, *112*(12), 1796–1808.

Weiss, E. P., Jordan, R. C., Frese, E. M., Albert, S. G., & Villareal, D. T. (2017). Effects of weight loss on lean mass, strength, bone, and aerobic capacity. *Medicine and Science in Sports and Exercise*, *49*(1), 206–217.

Weiss, E. P., Racette, S. B., Villareal, D. T., Fontana, L., Steger-May, K., Schechtman, K. B., … Holloszy, J. O. (2007). Lower extremity muscle size and strength and aerobic capacity decrease with caloric restriction but not with exercise-induced weight loss. *Journal of Applied Physiology*, *102*, 634–640.

Wente, W., Efanov, A. M., Brenner, M., Kharitonenkov, A., Köster, A., Sandusky, G. E., … Gromada, J. (2006). Fibroblast growth factor-21 improves pancreatic β-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. *Diabetes*, *55*(9), 2470–2478.

Westerbacka, J., Kolak, M., Kiviluoto, T., Arkkila, P., Sirén, J., Hamsten, A., … Yki-Järvinen, H. (2007). Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes*, *56*(11), 2759–2765.

Westerbacka, J., Lammi, K., Häkkinen, A. M., Rissanen, A., Salminen, I., Aro, A., & Yki-Järvinen, H. (2005). Dietary fat content modifies liver fat in overweight nondiabetic subjects. *Journal of Clinical Endocrinology and Metabolism*, *90*(5), 2804–2809.

Weston, K. S., Wisløff, U., & Coombes, J. S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *British Journal of Sports Medicine*, *48,* 1227–1234.

Whitlock, G., Lewington, S., Sherliker, P., Clarke, R., Emberson, J., Halsey, J., … Peto, R. (2009). Body-mass index and cause-specific mortality in 900 000 adults: Collaborative analyses of 57 prospective studies. *The Lancet*, *373*, 1083–1096.

Whyte, L. J., Gill, J. M. R., & Cathcart, A. J. (2010). Effect of 2 weeks of sprint interval training on health-related outcomes in sedentary overweight/obese men. *Metabolism: Clinical and Experimental*, *59*(10), 1421–1428.

Wildman, R. P., Muntner, P., Reynolds, K., McGinn, A. P., Rajpathak, S., Wylie-Rosett, J., & Sowers, M. R. (2008). The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering. *Arch Intern Med*, *168*(15), 1617–1624.

Williams, R., Aspinall, R., Bellis, M., Camps-walsh, G., Cramp, M., Dhawan, A., … Smith, T. (2014). Addressing liver disease in the UK : a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis. *The Lancet*, *384*, 1953–1997.

Williamson, R. M., Price, J. F., Glancy, S., Perry, E., Nee, L. D., Hayes, P. C., … Strachan, M. W. J. (2011). Prevalence of and risk factors for hepatic steatosis and nonalcoholic fatty liver disease in people with type 2 diabetes: The Edinburgh type 2 diabetes study. *Diabetes Care*, *34*(5), 1139–1144.

Willis, S. A., Sargeant, J. A., Stensel, D. J., & King, J. A. (2018). The effect of exercise intensity on circulating hepatokine concentrations in healthy men. *Obesity Facts*, *11*(Suppl 1), 37.

Wisløff, U., Støylen, A., Loennechen, J. P., Bruvold, M., Rognmo, Ø., Haram, P. M., … Skjærpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: A randomized study. *Circulation*, *115*(24), 3086–3094.

Wolfe, R. R., & Chinkes, D. L. (2005). Calculation of subtrate kinetics: single pool model. In R. R. Wolfe & D. L. Chinkes (Eds.), *Isotope tracers in metabolic research. Principles and practice of kinetic analysis* (2nd ed., pp. 21–50). New Jersey: John Wiley & Sons Ltd.

Wong, R. J., Cheung, R., & Ahmed, A. (2014). Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology*, *59*(6), 2188–2195.

Wong, V. W.-S., Chan, R. S.-M., Wong, G. L.-H., Cheung, B. H.-K., Chu, W. C.-W., Yeung, D. K.-W., … Chan, H. L.-Y. (2013). Community-based lifestyle modification programme for non-alcoholic fatty liver disease: a randomized controlled trial. *Journal of Hepatology*, *59*(3), 536–542.

Wood, K., Olive, B., LaValle, K., Thompson, H., Greer, K., & Astorino, T. (2016). Dissimilar physiological and perceptual responses between sprint interval training and high-intensity interval training. *Journal of Strength and Conditioning Research*, *30*(1), 244–250.

World Health Organisation. (2013). Declaration of Helsinki. Ethical principles for medical research involving human subjects. *The Journal of the American Medical Association*, *310*(20), 2191–2194.

Wu, H.-T., Ou, H.-Y., Hung, H.-C., Su, Y.-C., Lu, F.-H., Wu, J.-S., … Chang, C.-J. (2016). A novel hepatokine , HFREP1 , plays a crucial role in the development of insulin resistance and type 2 diabetes. *Diabetologia, 59*(8), 1732-1742.

Wu, H., Wu, M., Chen, Y., Allan, C. A., Phillips, D. J., & Hedger, M. P. (2012). Correlation between blood activin levels and clinical parameters of type 2 diabetes. *Experimental Diabetes Research*, *2012*, 1-9.

Wu, K.-T., Kuo, P.-L., Su, S.-B., Chen, Y.-Y., Yeh, M.-L., Huang, C.-I., … Wang, H.-Y. (2016). Nonalcoholic fatty liver disease severity is associated with the ratios of total cholesterol and triglycerides to high-density lipoprotein cholesterol. *Journal of Clinical Lipidology*, *10*(2), 420–425.

Xu, H., & Carrero, J. J. (2017). Insulin resistance in chronic kidney disease. *Nephrology*, *22*(Suppl 4), 31–34.

Xu, J., Lloyd, D. J., Hale, C., Stanislaus, S., Chen, M., Sivits, G., … Véniant, M. M. (2009). Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes*, *58*(1), 250–259.

Xu, J., Stanislaus, S., Chinookoswong, N., Lau, Y. Y., Hager, T., Patel, J., … Veniant, M. M. (2009). Acute glucose-lowering and insulin-sensitizing action of FGF21 in insulin-resistant mouse models--association with liver and adipose tissue effects. *American Journal of Physiology: Endocrinology and Metabolism*, *297*(5), E1105–E1114.

Xu, Y., Xu, M., Bi, Y., Song, A., Huang, Y., Liu, Y., … Ning, G. (2011). Serum fetuin-A is correlated with metabolic syndrome in middle-aged and elderly Chinese. *Atherosclerosis*, *216*(1), 180–186.

Yaden, B. C., Croy, J. E., Wang, Y., Wilson, J. M., Datta-Mannan, A., Shetler, P., … Krishnan, V. (2014). Follistatin: a novel therapeutic for the improvement of muscle regeneration. *The Journal of Pharmacology and Experimental Therapeutics*, *349*(2), 355–371.

Yamagoe, S., Mizuno, S., & Suzuki, K. (1998). Molecular cloning of human and bovine LECT2 having a neutrophil chemotactic activity and its specific expression in the liver. *Biochimica et Biophysica Acta*, *1396*(1), 105–113.

Yamagoe, S., Yamakawa, Y., Matsuo, Y., Minowada, J., Mizuno, S., & Suzuki, K. (1996). Purification and primary amino acid sequence of a novel neutrophil chemotactic factor LECT2. *Immunology Letters*, *52*(1), 9–13.

Yamaguchi, K., Yang, L., McCall, S., Huang, J., Xing, X. Y., Pandey, S. K., … Diehl, A. M. (2007). Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology*, *45*(6), 1366–1374.

Yamashita, H., Takenoshita, M., Sakurai, M., Bruick, R. K., Henzel, W. J., Shillinglaw, W., … Uyeda, K. (2001). A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver. *Proceedings of the National Academy of Sciences U S A*, *98*(16), 9116–9121.

Yang, S. J., Hong, H. C., Choi, H. Y., Yoo, H. J., Cho, G. J., Hwang, T. G., … Choi, K. M. (2011). Effects of a three-month combined exercise programme on fibroblast growth factor 21 and fetuin-A levels and arterial stiffness in obese women. *Clinical Endocrinology*, *75*(4), 464–469.

Yang, S. J., Hwang, S. Y., Choi, H. Y., Yoo, H. J., Seo, J. A., Kim, S. G., … Choi, K. M. (2011). Serum selenoprotein P levels in patients with type 2 diabetes and prediabetes: Implications for insulin resistance, inflammation, and atherosclerosis. *Journal of Clinical Endocrinology and Metabolism*, *96*(8), 1325–1329.

Yilmaz, Y., Eren, F., Yonal, O., Kurt, R., Aktas, B., Celikel, C. A., … Avsar, E. (2010). Increased serum FGF21 levels in patients with nonalcoholic fatty liver disease. *European Journal of Clinical Investigation*, *40*(10), 887–892.

Yilmaz, Y., Yonal, O., Kurt, R., Ari, F., Oral, A. Y., Celikel, C. A., … Kalayci, C. (2010). Serum fetuin A/alpha2HS-glycoprotein levels in patients with non-alcoholic fatty liver disease: relation with liver fibrosis. *Annals of Clinical Biochemistry*, *47*(6), 549–553.

Yndestad, A., Haukeland, J. W., Dahl, T. B., Bjøro, K., Gladhaug, I. P., Berge, C., … Aukrust, P. (2009). A complex role of activin A in non-alcoholic fatty liver disease. *The American Journal of Gastroenterology*, *104*(12), 2196–2205.

You, T., Berman, D. M., Ryan, A. S., & Nicklas, B. J. (2004). Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. *Journal of Clinical Endocrinology and Metabolism*, *89*(4), 1739–1746.

Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., & Wymer, M. (2016). Global epidemiology of non-alcoholic fatty liver disease – meta-analytic assessment of prevalence, incidence and outcomes. *Hepatology*, *64*(1), 73–84.

Yumuk, V., Tsigos, C., Fried, M., Schindler, K., Busetto, L., Micic, D., & Toplak, H. (2015). European guidelines for obesity management in adults. *Obesity Facts*, *8*(6), 402–424.

Zein, C. O., Yerian, L. M., Gogate, P., Lopez, R., Kirwan, J. P., Feldstein, A. E., & McCullough, A. J. (2011). Pentoxifylline improves nonalcoholic steatohepatitis: A randomized placebo-controlled trial. *Hepatology*, *54*(5), 1610–1619.

Zelber-Sagi, S., Buch, A., Yeshua, H., Vaisman, N., Webb, M., Harari, G., … Shibolet, O. (2014). Effect of resistance training on non-alcoholic fatty-liver disease a randomized-clinical trial. *World Journal of Gastroenterology*, *20*(15), 4382–4392.

Zelber-Sagi, S., Kessler, A., Brazowsky, E., Webb, M., Lurie, Y., Santo, M., … Oren, R. (2006). A double-blind randomized placebo-controlled trial of orlistat for the treatment of nonalcoholic fatty liver disease. *Clinical Gastroenterology and Hepatology*, *4*(5), 639–644.

Zelber-Sagi, S., Nitzan-Kaluski, D., Goldsmith, R., Webb, M., Blendis, L., Halpern, Z., & Oren, R. (2007). Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): A population based study. *Journal of Hepatology*, *47*(5), 711–717.

Zelber-Sagi, S., Ratziu, V., & Oren, R. (2011). Nutrition and physical activity in NAFLD: An overview of the epidemiological evidence. *World Journal of Gastroenterology*, *17*(29), 3377–3389.

Zhang, H.-J., He, J., Pan, L.-L., Ma, Z.-M., Han, C.-K., Chen, C.-S., … Li, X.-Y. (2016). Effects of moderate and vigorous exercise on nonalcoholic fatty liver disease. The *Journal of the American Medical Association. Internal Medicine*, *176*(8), 1074–1082.

Zhang, X., Yeung, D. C. Y., Karpisek, M., Stejskal, D., Zhou, Z., Liu, F., … Lam, K. S. L. (2008). Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes*, *57*(5), 1246–1253.

Zhao, C., Qiao, C., Tang, R.-H., Jiang, J., Li, J., Martin, C. B., … Xiao, X. (2015). Overcoming insulin insufficiency by forced follistatin expression in β-cells of db/db mice. *Molecular Therapy : The Journal of the American Society of Gene Therapy*, *23*(5), 866–874.

Ziogas, G., Thomas, T., & Harris, W. (1997). Exercise training, postprandial hypertriglyceridemia, and LDL subfraction distribution. *Medicine and Science in Sports and Exercise*, *29*(8), 986–991.

CHAPTER 10

APPENDICES

10.1 Appendix I: *Applied Physiology Nutrition and Metabolism*, featured content May 2018

10.2 Appendix II: Ethical and sponsor approvals

10.2.1 Chapter 4: Research ethics committee approval

Re-issue Further Information Favourable Opinion, 22 October 2013

NHS
Health Research Authority
NRES Committee East Midlands - Nottingham 1
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 883 9390

07 October 2013

Professor Melanie Davies
Leicester Diabetes Centre
Leicester General Hospital
Leicester
LE5 4PW

Dear Professor Davies,

Study title:	**The acute effects of exercise on appetite regulatory hormones, appetite perceptions and ad libitum energy intake in lean vs. obese men and women.**
REC reference:	**13/EM/0290**
Protocol number:	**1**
IRAS project ID:	**124117**

Thank you for your letter of 26 September 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Assistant Rebecca Morledge, NRESCommittee.EastMidlands-Nottingham1@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Advertisement	1	28 February 2013
Evidence of insurance or indemnity		15 May 2013
Investigator CV	Melanie Jane Davies	26 June 2013
Investigator CV	Jessica Anne Douglas	13 June 2013
Letter from Statistician		24 June 2013
Letter of invitation to participant	1	13 June 2013
Other: Preliminary Appointment Letter	1	13 June 2013
Other: Familiarisation Appointment Letter	1	13 June 2013
Other: Exercise Trial Appointment Letter	1	13 June 2013
Other: Resting Trial Appointment Letter	1	13 June 2013
Other: Letter from funder		12 June 2013
Other: Joint Office Sponsor Peer Review Form		12 February 2013
Other: Food Record Diary	1	12 June 2013
Other: Reply Slip	1	27 March 2013
Other: GP Results Letter	2	26 September 2013
Other: Participant Results Letter	1	26 September 2013
Participant Consent Form	2	26 September 2013
Participant Information Sheet	2	26 September 2013
Protocol	1	13 June 2013
Questionnaire: INTAKE IPAQ	1	13 June 2013
Questionnaire: INTAKE PARQ	1	28 February 2013
Questionnaire: Three Factor Eating Questionnaire	1	28 February 2013
Questionnaire: Health Screen	1	13 June 2013
Questionnaire: Food Preference	1	28 February 2013
REC application	124117/472008/1/239	02 July 2013
Response to Request for Further Information		26 September 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/EM/0290	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely,

pp

Mr Robert Johnson
Chair

Email:NRESCommittee.EastMidlands-Nottingham1@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Ms Carolyn Maloney, University Hospitals Leicester NHS Trust*
James King

10.2.2 Chapter 4: R&I approval

University Hospitals of Leicester NHS
NHS Trust

DIRECTORATE OF RESEARCH & DEVELOPMENT

Director: **Professor N Brunskill**
Assistant Director: **Dr David Hetmanski**
R&D Manager: **Carolyn Maloney**

Research & Development Office
Leicester General Hospital
Gwendolen Road
Leicester
LE5 4PW

Direct Dial: (0116) 258 8351
Fax No: (0116) 258 4226

26th March 2014

Professor Melanie Davies
Leicester Diabetes Centre
Leicester General Hospital
Leicester
LE5 4PW

Dear Professor Melanie Davies

Ref: **UHL 124117**
Title: **The acute effects of exercise on appetite regulatory hormones, appetite perceptions and ad libitum energy intake in lean vs. obese men and women.**
Project Status: **Approved**
End Date: **07/12/2015**

I am pleased to confirm that with effect from the date of this letter, the above study has Trust Research & Development permission to commence at University Hospitals of Leicester NHS Trust. The research must be conducted in line with the Protocol and fulfil any contractual obligations agreed with the Sponsor. If you identify any issues during the course of your research that are likely to affect these obligations you must contact the R&D Office.

In order for the UHL Trust to comply with targets set by the Department of Health through the 'Plan for Growth', there is an expectation that the first patient will be recruited within 30 days of the date of this letter. If there is likely to be a problem achieving this target, please contact the office as soon as possible. You will be asked to provide the date of the first patient recruited in due course. In addition, the Title, REC Reference number, local target recruitment and actual recruitment for this study will be published on a quarterly basis on the UHL Trust external website.

All documents received by this office have been reviewed and form part of the approval. The documents received and approved are as follows:

Document Title	Version	Date	REC Approval
REC favourable opinion letter	N/A	07.10.13	N/A
1.Advertisement	1	28.02.13	07.10.13
2.Evidence of insurance or indemnity	N/A	15.05.13	07.10.13
3.Invesigator CV	MJ Davies	26.06.13	07.10.13
4.Investigator CV	JA Douglas	13.06.13	07.10.13
5.Letter from Statistician	N/A	24.06.13	07.10.13
6.Letter of invitation to participant	1	13.06.13	07.10.13
7.Preliminary appointment letter	1	13.06.13	07.10.13
8.Familiarisaton appointment letter	1	13.06.13	07.10.13
9.Exercise Trial appointment letter	1	13.06.13	07.10.13

Version 11, 16/10/2012

10.Resting Trial appointment letter	1	13.06.13	07.10.13
11.Letter from funder	N/A	12.06.13	07.10.13
12.Joint Office Sponsor Peer Review Form	N/A	12.02.13	07.10.13
13.Food Record Diary	1	12.06.13	07.10.13
14.Reply Slip	1	27.03.13	07.10.13
15.GP Results Letter	2	26.09.13	07.10.13
16.Participants Results Letter	1	26.09.13	07.10.13
17.Particpant Consent Form	2	26.09.13	07.10.13
18.Participant Information Sheet	2	26.09.13	07.10.13
19.Protocol	1	13.06.13	07.10.13
20. Questionnaire: INTAKE IPAQ	1	13.06.13	07.10.13
21. Questionnaire : INTAKE PARQ	1	28.02.13	07.10.13
22. Questionnaire: Three Factor Eating	1	28.02.13	07.10.13
23. Questionnaire: Health Screen	1	13.06.13	07.10.13
24. Questionnaire: Food Preference	1	28.02.13	07.10.13

Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments should be followed. Failure to do so may invalidate the approval of the study at this trust.

Undertaking research in the NHS comes with a range of regulatory responsibilities. Please ensure that you and your research team are familiar with, and understand the roles and responsibilities both collectively and individually.

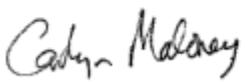
Documents listing the roles and responsibilities for all individuals involved in research can be found on the R&D pages of the Public Website. It is important that you familiarise yourself with the Standard Operating Procedures, Policies and all other relevant documents which can be located by visiting www.leicestershospitals.nhs.uk/aboutus/education-and-research

The R&D Office is keen to support and facilitate research where ever possible. If you have any questions regarding this or other research you wish to undertake in the Trust, please contact this office. Our contact details are provided on the attached sheet.

This study has been reviewed and processed by the Leicestershire, Northamptonshire & Rutland Comprehensive Local Research Network (LNR CLRN) using the Coordinated System for gaining Trust Permission (CSP). If you require any further information on the approval of this study please contact the LNR CLRN office on 0116 258 6185 making reference to the CSP number which is located at the top of this letter.

We wish you every success with your research.

Yours sincerely



Carolyn Maloney
R&D Manager

Encs: .R&D Office Contact Information

10.2.3 Chapter 4: Sponsor approval

University of Leicester

College of Medicine, Biological Sciences & Psychology
University of Leicester
Research Governance Office
Academic Department, Ground Floor
Leicester General Hospital
Gwendolen Road
Leicester, LE5 4PW
Email: uolsponsor@le.ac.uk
Tel: 0116 258 4099/258 4867

26th March 2014

Professor Melanie Davies
University of Leicester
Leicester Diabetes Centre
Leicester General Hospital

Dear Professor Davies

Study: 0388
The acute effects of exercise on appetite regulatory hormones, appetite perceptions and ad libitum energy intake in lean vs. obese men and women.
End of Study Date: 07/12/2015

I am pleased to advise you that following confirmation of a Favourable Opinion from an Ethics Committee, NHS Trust R&D Approval and where relevant regulatory authority agreements have been received, the University are able to confirm sponsorship for the above research.

Please note you are required to notify the Sponsor and provide copies of:

- Changes in personnel to the Study
- Changes to the end date
- All substantial amendments and provisional and favourable opinions
- All minor amendments
- All serious adverse events (SAEs) and SUSARS
- Annual progress reports
- Annual MHRA (DSUR) safety reports (if applicable)
- End of study declaration form
- Notifications of significant breaches of Good Clinical Practices (GCP)or Protocol

Please copy the Sponsor into all correspondence and emails by using uolsponsor@le.ac.uk.

I would like to wish you well with your study and if you require further information or guidance please do not hesitate to contact me.

Yours sincerely

W. Gamble

Mrs Wendy Gamble
Research Governance Manager

10.2.4 Chapter 5: Research ethics committee approval

NHS
Health Research Authority
NRES Committee East Midlands - Nottingham 1
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 8839697

28 July 2015

Professor Melanie Davies
Leicester Diabetes Centre
Leicester General Hospital
Leicester
LE5 4PW

Dear Professor Davies,

Study title:	**The effect of a single bout of high-intensity interval training on glucose responses in white European and south Asian patients at risk of type 2 diabetes.**
REC reference:	**15/EM/0259**
Protocol number:	**UNOLE 0521**
IRAS project ID:	**167328**

Thank you for your letter of 15th July 2015, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager, Ms Rachel Nelson, at NRESCommittee.EastMidlands-Nottingham1@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the

study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet completed any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [GO for IT Study Poster]	2	11 June 2015
Covering letter on headed paper [REC review response letter]	1	08 July 2015
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [UoL letter of indemnity]	1	06 May 2015
GP/consultant information sheets or letters [GO for IT GPIL]	1	20 May 2015
Instructions for use of medical device [GO for IT CGM instruction leaflet]	1	06 July 2015
IRAS Checklist XML [Checklist_15072015]		15 July 2015
Letter from funder [LDC funding confirmation]		02 April 2015
Letters of invitation to participant [GO for IT database PIL]	1	20 March 2015
Letters of invitation to participant [GO for IT GP PIL]		
Non-validated questionnaire [GO for IT PA QA]	1	20 March 2015
Non-validated questionnaire [GO for IT Pre-screening QA]	1	20 March 2015
Non-validated questionnaire [GO for IT Food Diary]	1	20 March 2015
Participant consent form [GO for IT ICF]	2	23 June 2015
Participant information sheet (PIS) [GO for IT PIS]	2	23 June 2015
REC Application Form [REC_Form_26052015]		26 May 2015
Referee's report or other scientific critique report [GO for IT Peer Review JG]		21 March 2015
Referee's report or other scientific critique report [GO for IT Peer Review KT]		
Research protocol or project proposal [GO for IT Trial Protocol V1 April 15]	1	24 April 2015
Research protocol or project proposal [GO for IT Trial Protocol V1 April 15]	2	06 July 2015
Summary CV for Chief Investigator (CI) [MJD CI CV]	1	12 March 2015
Summary CV for student [Jelleyman CV 2015]	2	23 June 2015
Summary CV for supervisor (student research) [Yates SUpervisor CV]		04 January 2015
Validated questionnaire [Three factor eating QA]		
Validated questionnaire [Mood, Affect & Sleepiness QA]		
Validated questionnaire [Satiety QA]		

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

15/EM/0259	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely,

pp

Dr Carl Edwards
Chair

Email: NRESCommittee.EastMidlands-Nottingham1@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Ms. Wendy Gamble*

Mrs. Carolyn Maloney

10.2.5 Chapter 5: R&I approval

RESEARCH & INNOVATION

University Hospitals of Leicester NHS
NHS Trust

DIRECTORATE OF RESEARCH & INNOVATION

Director: **Professor Nigel Brunskill**
Assistant Director: **Dr David Hetmanski**
Head of Research Operations: **Carolyn Maloney**

Research & Innovation Office
Leicester General Hospital
Gwendolen Road
Leicester
LE5 4PW

Direct Dial: (0116) 258 8351
Fax No: (0116) 258 4226

22nd October 2015

Dr Tom Yates
Leicester Diabetes Centre
Leicester General Hospital
Leicester
LE5 4PW

Dear Dr Tom Yates

Ref: **UHL 167328**
Title: **The effect of a single bout of high intensity interval training on glucose responses in white European and south Asian patients at risk of type 2 diabetes.**
Project Status: **Approved**
End Date: **30/09/2016**

Date of Valid Application: 20th October 2015
Days remaining to recruit first patient: 68 days

I am pleased to confirm that with effect from the date of this letter, the above study has Trust Research & Development permission to commence at University Hospitals of Leicester NHS Trust. The research must be conducted in line with the Protocol and fulfil any contractual obligations agreed between UHL & the Sponsor. If you identify any issues during the course of your research that are likely to affect these obligations you must contact the R&I Office.

In order for the UHL Trust to comply with targets set by the Department of Health through the 'Plan for Growth', there is an expectation that the first patient will be recruited within 70 days of receipt of a Valid Application. The date that a Valid application was received is detailed above, along with the days remaining to recruit your first patient. **It is essential that you notify the UHL Data Management Team as soon as you have recruited your first patient to the study either by email to RIData@uhl-tr.nhs.uk or by phone 0116 258 4573.**

If we have not heard from you within the specified time period we will contact you not only to collect the data, but also to record any issues that may have arisen to prevent you from achieving this target. It is essential that you get in touch with us if there is likely to be a problem in achieving this target so that we can discuss potential solutions. The Trust is contractually

Version 14, 03/06/2015

obliged to meet the 70 day target and if an adequate reason acceptable to the NIHR has not been submitted to explain the issues preventing the recruitment of your first participant, the Trust will be financially penalised.

In addition, we are required to publish the Title, REC Reference number, local target recruitment and actual recruitment as well as 70 days data for this study on a quarterly basis on the UHL publicly accessed website.

All documents received by this office have been reviewed and form part of the approval. The documents received and approved are as follows:

Document Title	Version	Date	REC Approval
REC favourable opinion letter	N/A	28.07.15	N/A
Copies of advertisement materials for research participants [GO for IT Study Poster]	2	11.06.15	28.07.15
GP/consultant information sheets or letters [GO for IT GPIL]	1	20.05.15	28.07.15
Instructions for use of medical device [GO for IT CGM instruction leaflet]	1	06.07.15	28.07.15
Letters of invitation to participant [GO for IT database PIL]	1	20.03.15	28.07.15
Letters of invitation to participant [GO for IT GP PIL]			28.07.15
Non-validated questionnaire [GO for IT PA QA]	1	20.03.15	28.07.15
Non-validated questionnaire [GO for IT Pre-screening QA]	1	20.03.15	28.07.15
Non-validated questionnaire [GO for IT Food Diary]	1	20.03.15	28.07.15
Participant consent form [GO for IT ICF]	2	23.06.15	28.07.15
Participant information sheet (PIS) [GO for IT PIS]	2	23.06.15	28.07.15
Research protocol or project proposal [GO for IT Trial Protocol)	2	06.07.15	28.07.15
Validated questionnaire [Three factor eating QA]	N/A	N/A	28.07.15
Validated questionnaire [Mood, Affect & Sleepiness QA]	N/A	N/A	28.07.15
Validated questionnaire [Satiety QA]	N/A	N/A	28.07.15

Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments should be followed. Failure to do so may invalidate the approval of the study at this trust.

Undertaking research in the NHS comes with a range of regulatory responsibilities. Please ensure that you and your research team are familiar with, and understand the roles and responsibilities both collectively and individually.

Documents listing the roles and responsibilities for all individuals involved in research can be found on the R&I pages of the Public Website. It is important that you familiarise yourself with the Standard Operating Procedures, Policies and all other relevant documents which can be located by visiting www.leicestershospitals.nhs.uk/aboutus/education-and-research

RESEARCH & INNOVATION

University Hospitals of Leicester NHS
NHS Trust

The R&I Office is keen to support and facilitate research where ever possible. If you have any questions regarding this or other research you wish to undertake in the Trust, please contact this office. Our contact details are provided on the attached sheet.

This study has been reviewed and processed by the East Midlands Clinical Research Network (EM CRN) (Leicester Office) using the Coordinated System for gaining Trust Permission (CSP). If you require any further information on the approval of this study please contact the EM CRN office on 0116 258 6185 making reference to the CSP number which is located at the top of this letter.

We wish you every success with your research.

Yours sincerely

Carolyn Maloney

Carolyn Maloney
Head of Research Operations

Encs: .R&I Office Contact Information

10.2.6 Chapter 5: Sponsor approval

UNIVERSITY OF LEICESTER

College of Medicine, Biological Sciences & Psychology
University of Leicester
Research Governance Office
Academic Department, Ground Floor
Leicester General Hospital
Gwendolen Road
Leicester, LE5 4PW
Email: uolsponsor@le.ac.uk
Tel: 0116 258 4099/258 4867

12 November 2015

Dr Thomas Yates
Leicester Diabetes Centre
Leicester General Hospital
LE5 4PW

Dear Dr Thomas Yates

Study: **UNOLE 0521**
Title: **The effect of a single bout of high intensity interval training on glucose responses in white European and South Asian patients at risk of Type 2 Diabetes**
Study Status: **Approved**
End Date: **30/09/2016**

I am pleased to advise you that following confirmation of a Favourable Opinion from an Ethics Committee, NHS Trust R&D Approval and where relevant regulatory authority agreements have been received, the University are able to confirm sponsorship for the above research.

Please note you are required to notify the Sponsor and provide copies of:

- Changes in personnel to the Study
- Changes to the end date
- All substantial amendments and provisional and favourable opinions
- All minor amendments
- All serious adverse events (SAEs) and SUSARS
- Annual progress reports
- Annual MHRA (DSUR) safety reports (if applicable)
- End of study declaration form
- Notifications of significant breaches of Good Clinical Practices (GCP)or Protocol

Please copy the Sponsor into all correspondence and emails by using uolsponsor@le.ac.uk.

I would like to wish you well with your study and if you require further information or guidance please do not hesitate to contact me.

Yours sincerely

Mrs Wendy Gamble
Research Governance Manager

10.2.7 Chapter 6: Research ethics committee approvals

Ref No: R14-P39

LOUGHBOROUGH UNIVERSITY
ETHICS APPROVALS (HUMAN PARTICIPANTS) SUB-COMMITTEE

RESEARCH PROPOSAL
INVOLVING HUMAN PARTICIPANTS

Title:	High Intensity Training & Insulin Sensitivity: A Tissue Specific Assessment
Applicant:	Prof M Nimmo, Z Amir, Dr J King, Dr J Viana, L Simpson, Prof P Gowland, Prof I Macdonald
Department:	SSEHS
Date of clearance:	26 February 2014

Comments of the Sub-Committee:

The Sub-Committee agreed to issue conditional approval, subject to the following conditions:

- That confirmation was provided that the University of Nottingham had also provided ethical approval for this study.
- That a return visit health check was used for each additional visit.
- That the advertisement was amended to remove the amount of money provided to the participants.

Direct line/e-mail
+44 (0) 115 8232561
Louise.Sabir@nottingham.ac.uk

Faculty of Medicine and Health Sciences

Research Ethics Committee
Division of Respiratory Medicine
D Floor, South Block
Queen's Medical Centre
Nottingham University Hospitals
Nottingham
NG7 2UH

7th March 2014

Professor Penny Gowland
Professor of Physics
School of Physics & Astronomy
Sir Peter Mansfield Magnetic Resonance Centre
University Park Campus
University of Nottingham
NG7 2RD

Dear Professor Gowland

Ethics Ref No: A28022014 SPMMRC Lboro Ref: R14-P39 – **please always quote**
Study Title: High Intensity Training & Insulin Sensitivity: A Tissue Specific Assessment.
Chief Researcher/Supervisor: Professor M Nimmo, School of Sport, Exercise and Health Sciences (SSEHS), University of Loughborough.
Lead Researcher/Student: Zainab Amir, PhD Student, SSEHS, University of Loughborough
Co-Researchers/Supervisor: Dr James King, Senior Research Associate, Dr Joao Viana, Research Associate, SSEHS, University of Loughborough, Professor Penny Gowland, SPMMRC, School of Physics and Astronomy Dr Liz Simpson, Senior Experimental Officer, Professor Ian Macdonald, Metabolic Physiology School of Life Sciences Professor Guruprasad Aithal, Hepatology School of Medicine, University of Nottingham.
Duration of Study: March 2014-March 2016 2 yrs **No of Subjects:** 8

Thank you for your letter and application dated 28th February 2014 for a study which was given conditional approval by the Loughborough University Ethics Approvals (Human Participants) Sub-Committee Ref No: R14-P39 on 26th February 2014 and the following documents were received:

1. Letter of conditional Approval dated 26th February 2014 Loughborough University Ethics Approvals (Human Participants) Sub-Committee Ref No: R14-P39.

2. Loughborough University Ethics Approvals (Human Participants) Sub-Committee: Research Proposal for Studies involving Human Participants form dated 05/02/2014.

These have been reviewed and are satisfactory and the study has been approved.

Please can you submit copies of the Participant Information Sheet, Consent Form, Health Screen Questionnaire, Questionnaires and Advertisement/Recruitment material which have been approved for our files please.

Approval is given on the understanding that the Conditions of Approval set out below are followed.

1. You must follow the protocol agreed and inform the Committee of any changes using a notification of amendment form (please request a form).

2. You must notify the Chair of any serious or unexpected event.

3. This study is approved for the period of active recruitment requested. The Committee also provides a further 5 year approval for any necessary work to be performed on the study which may arise in the process of publication and peer review.

4. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

Dr Clodagh Dugdale
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

10.2.8 Chapter 6: Confirmation of amendment to change lead research student

From: Jacqueline Green
Sent: 20 October 2014 12:09
To: James King
Cc: Jack Sargeant
Subject: RE: R14-P39

Hi James

I can confirm that R14-P39 has been amended as your email below and has full ethical approval.

Kind regards

Jackie

Ms Jacqueline Green
Secretary, Ethics Approvals (Human Participants) Sub-Committee
Research Office
Tel: 01509 222423
Email: J.A.Green@lboro.ac.uk

From: James King
Sent: 20 October 2014 10:04
To: Jacqueline Green
Cc: Jack Sargeant
Subject: R14-P39

Dear Jackie,

We would like to make two small amendments to the ethics for the Project R14-P39 (High Intensity Training and Insulin Sensitivity: A Tissue Specific Assessment).

1) Jack Sargeant to be added to the ethics and Zainab Amir removed – Jack is the new PhD student leading the project who is replacing Zainab who left us.
2) We would like to change the HIT exercise protocol; from the 1 min on/min off protocol (repeated 10 times) to 30 second maximal bursts with 4 min recovery (repeated up to 6 times per session). The background to this second change is that there are several 'HIT' protocols implemented within the literature and our group have used the 1 min on/off protocol recently and not seen any beneficial changes. From the literature the 30 second burst protocol seems to be highly effective at improving the primary outcome in this study.

Please can you confirm whether these changes are ok?

Many thanks

James

James King PhD
Senior Research Associate
Leicester-Loughborough Diet, Lifestyle & Physical Activity BRU
School of Sport, Exercise and Health Sciences

1

10.3 Appendix III: Details of risk definition and screening procedures for inactive individuals as detailed in the Leicester-Loughborough BRU risk SOP/RA for exercise testing.

SECTION 2 – RISK DEFINITION AND MANAGEMENT

Extensive participant pre-participation evaluation must be undertaken before individuals participate in exercise testing or training in any research encompassed within the Leicester-Loughborough Diet, Lifestyle & Physical Activity BRU. This applies to all participants, regardless of age.

DISCUSSION OF CRITERIA – ACTIVITY STATUS

- DEFINITION OF ACTIVITY STATUS

The EACPR identifies two separate pre-exercise evaluation work flows which establish more stringent pre-exercise evaluation procedures for those who are currently inactive compared with those who are active. This reflects the greater risk in those who are routinely inactive (Siscovick et al, 1984; Willich et al, 1993; Mittleman et al, 1994). The EACPR classifies participants' activity status based on whether they perform more or less than 2 MET-h/wk of physical activity. In essence, this definition of activity only categorises individuals as inactive if they habitually undertake a very low amount of physical activity. In addition, the risk of an adverse event is highly dependent on whether or not participants engage in regular vigorous physical activity; habitual vigorous physical activity (> 6 METs) substantially reduces the risk of sudden cardiac death during exercise (Siscovick et al, 1984; Willich et al, 1993; Mittleman et al, 1994). Therefore, a more robust definition of activity status is proposed here. Only individuals habitually performing exercise at a vigorous intensity on three or more days per week for a period of at least 20 minutes will be classified as active. Published data detailing the MET equivalents of diverse activities (Ainsworth et al, 2000) will be used to determine the intensity of activities reported by prospective participants.

In instances where there is ambiguity in the categorisation of an individual a judgement will be made by the responsible investigator. A hard copy of the questionnaire used to classify activity status can be found in Appendix 1. Appendixes 2-6 contain all of the forms needed for pre-exercise evaluation.

DISCUSSION OF CRITERIA – EXERCISE INTENSITY

- STRATIFICATION FOR THOSE WHO ARE INACTIVE/UNTRAINED (FIGURE 1)

The risk stratification algorithm will be dependent on the nature of the physical activity protocol proposed for each study.

Light intensity exercise (see Figure 1): Individuals due to engage in light intensity exercise (identified as < 3 METs) must first complete the Physical Activity Readiness Questionnaire (PAR-Q). If responses to this questionnaire are negative (individuals answer no to all seven questions) individuals are eligible to participate in an exercise intervention involving low-intensity exercise without further evaluation. One or more positive response dictates that further evaluation is necessary, which in the first instance, should involve consultation with a physician (which could be either the individual's GP or a BRU physician). At this point a judgment must be made by the physician/GP on whether the issue identified by the PAR-Q necessitates further in-depth evaluation (medical history, physical exam, risk SCORE assessment and 12 lead resting ECG) before the individual can take part in light intensity exercise. If the issue identified by the PAR-Q is deemed to be insufficient to warrant such further evaluation then the physician/GP can give permission for the individual to participate in light intensity exercise.

Moderate/Vigorous intensity exercise (see Figure 1): Inactive/untrained individuals being recruited to studies involving moderate (3-6 METs) or high (> 6 METs) intensity exercise must undergo a detailed stepped evaluation before participation. This evaluation should begin with completion of the PAR-Q, a medical history, physical exam, risk SCORE assessment, and 12 lead resting ECG. Following this, individuals will be classified into one of 3 groups which will determine whether nor not an additional exercise ECG is needed and where this should be conducted. Definitions of risk category are highlighted below and shown in Figure 2.

Low risk

Low risk individuals are those that are negative for all assessment criteria (PAR-Q, history of CVD or other chronic disease, physical exam, Risk SCORE, resting ECG). Low risk individuals will be eligible to participate in exercise training without further assessment

Moderate risk

Those failing to meet the criteria for low risk, but are outside the criteria for high risk. Moderate risk indicates the need to conduct an exercise ECG, which can be undertaken in any setting, subject to the presence of suitable trained individuals and resuscitation equipment and processes.

High risk

Those with any of the following criteria will be classified as high risk

- Those with a diagnosed chronic disease
- Those with a 10 year risk of CVD of $\geq$ 10%
- Raised total cholesterol (> 8 mmol/L), LDL-C (> 6mmol/L)
- Raised blood pressure (180/110 mmHG)
- Individuals with a strong family history of CVD in first degree relatives under 50 years of age
- Individuals with a BMI > 35 kg/m^2

High risk indicates the need for an exercise ECG which, due to the high risk nature of the individuals, should to be undertaken in a clinical setting by trained staff with access to a resuscitation team and post arrest management.

REFERENCES

Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett DR Jr, Schmitz KH, Emplaincourt PO, Jacobs DR Jr, Leon AS. (2000). Compendium of physical activities: an update of activity codes and MET intensities. *(Medicine and Science in Sports and Exercise)* 32:S498-504

American College of Sports Medicine (2010). ACSM's guidelines for Exercise Testing and Prescription (eighth edition). Lippincott, Williams & Wilkins. Philadelphia, PA.

Borjesson et al (2011). Cardiovascular evaluation of middle-aged/senior individuals engaged in leisure-time sport activities: position stand from the sections of exercise physiology and sports cardiology of the European Association of Cardiology Prevention and Rehabilitation. (*European Journal of Cardiovascular Prevention and Rehabilitation*) 18(3): 446-458

Black MM, Gensini G, and Black A. (1975). Exertion and Acute Coronary Artery Injury. Angiology 26: 759-783.

Ciampricotti R, Deckers JW, Taverne R, el Gamal M, Relik-van Wely L, and Pool J. (1994). Characteristics of conditioned and sedentary men with acute coronary syndromes. (*American Journal of Cardiology*) 1: 219-222.

Corrado D, Basso C, Schiavon M, and Thiene G. (1998). Screening for Hypertrophic Cardiomyopathy in Young Athletes. (*New England Journal of Medicine*) 339: 364-369.

Department of Health (2011) Physical Activity Guidelines for Adults (19-64 years) Fact Sheet 4. Accessed at https://www.gov.uk/government/publications/uk-physical-activity-guidelines on 11.4.13

Giri S, Thompson PD, Kiernan FJ, Clive J, Fram DB, Mitchel JF, Hirst JA, McKay RG, and Waters DD. (1999). Clinical and Angiographic Characteristics of Exertion-Related Acute Myocardial Infarction. (*Journal of the American Medical Association*) 282: 1731-1736.

Hammoudeh AJ, and Haft JI. (1996). Coronary-plaque rupture in acute coronary syndromes triggered by snow shoveling. N Engl J Med 335: 2001.

Knight JA, Laubach CA, Butcher RJ, and Menapace FJ. (1995). Supervision of clinical exercise testing by exercise physiologists. Am J Cardiol 75: 390-391.

Kragel AH, and Roberts WC. (1988). Sudden death and cardiomegaly unassociated with coronary, valvular, congenital or specific myocardial disease. (*American Journal of Cardiology*) 6: 659-660.

Mittleman MA, Maclure M, Tofler GH, Sherwood JB, Goldberg RJ, Muller JE, and The Determinants of Myocardial Infarction Onset Study I. (1993). Triggering of Acute Myocardial Infarction by Heavy Physical Exertion -- Protection against Triggering by Regular Exertion. N Engl J Med 329: 1677-1683..

Siscovick DS, Weiss NS, Fletcher RH, and Lasky T. (1984). The incidence of primary cardiac arrest during vigorous exercise. NEJM 4: 874-877.

Thompson PD, Stern MP, Williams P, Duncan K, Haskell WL, and Wood PD. (1979). Death during jogging or running. A study of 18 cases. Journal of the (*American Medical Association*) 21: 1265-1267.

Willich SN, Lewis M, Lowel H, Arntz H, Schubert F, Schroder R. (1993). Physical Exertion as a trigger of acute myocardial infarction. (*The New England Journal of Medicine*) 329: 1684-1690.

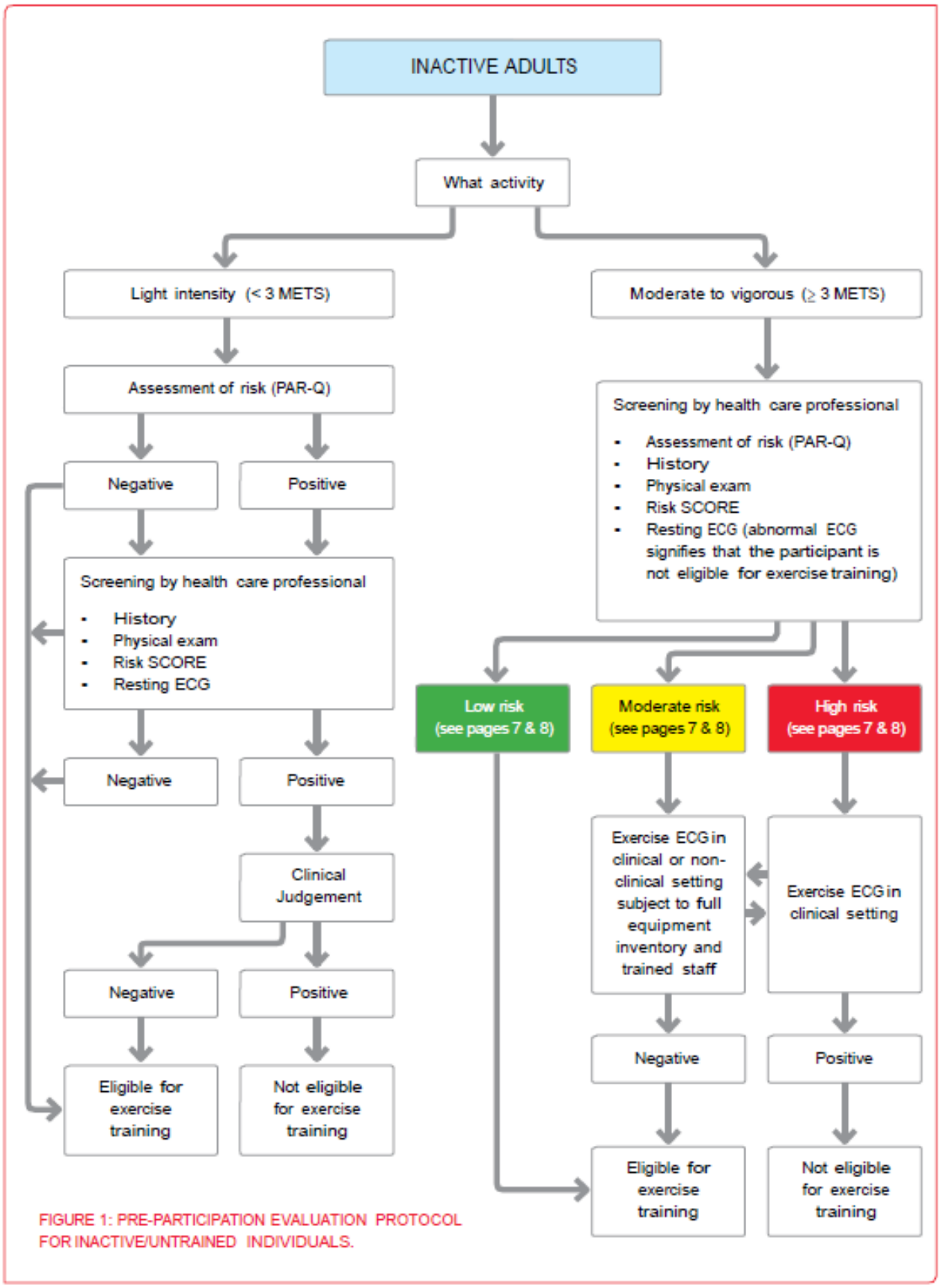


FIGURE 1: PRE-PARTICIPATION EVALUATION PROTOCOL FOR INACTIVE/UNTRAINED INDIVIDUALS.

10.4 Appendix IV: The international physical activity questionnaire (short version).

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRES

IPAQ: SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken in 12 countries (14 sites) across 6 continents during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages. IPAQ is suitable for use in regional, national and international monitoring and surveillance systems and for use in research projects and public health program planning and evaluation. International collaboration on IPAQ is on-going and an international prevalence study is under development.

Using IPAQ

Worldwide use of the IPAQ instruments for monitoring and research purposes is encouraged.

It is strongly recommended, to ensure data quality and comparability and to facilitate the development of an international database on health-related physical activity, that

- no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments,
- if additional questions on physical activity are needed they should follow the IPAQ items,
- translations are undertaken using the prescribed back translation methods (see website)
- new translated versions of IPAQ be made available to others via the web site to avoid duplication of effort and different versions in the same language,
- a copy of IPAQ data from representative samples at national, state or regional level be provided to the IPAQ data storage center for future collaborative use (with permission) by those who contribute.

More Information

Two scientific publications presenting the methods and the pooled results from the IPAQ reliability and validity study are due out in 2002.

More detailed information on the IPAQ process, the research methods used in the development of the IPAQ instruments, the use of IPAQ, the published papers and abstracts and the on-going international collaboration is available on the IPAQ web-site.

www.ipaq.ki.se

This is the final SHORT LAST 7 DAYS SELF-ADMINISTERED version of IPAQ from the 2000/01 Reliability and Validity Study. Completed May 2001.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

IPAQ: SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS

NOTE: EXAMPLES OF ACTIVITIES MAY BE REPLACED BY CULTURALLY RELEVANT EXAMPLES WITH THE SAME METS VALUES (SEE AINSWORTH *ET AL*., 2000).

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. This is part of a large study being conducted in many countries around the world. Your answers will help us to understand how active we are compared with people in other countries.

The questions are about the time you spent being physically active in the last 7 days. They include questions about activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Your answers are important.

Please answer each question even if you do not consider yourself to be an active person.

THANK YOU FOR PARTICIPATING.

In answering the following questions,

- **vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder that normal.

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

This is the final SHORT LAST 7 DAYS SELF-ADMINISTERED version of IPAQ from the 2000/01 Reliability and Validity Study. Completed May 2001.

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

Think about *only* those physical activities that you did for at least 10 minutes at a time.

_____ **days per week** ⇨

1b. How much time in total did you usually spend on one of those days doing vigorous physical activities*?*

_____ **hours** _____ **minutes**

or

☐ **none**

2a. Again, think *only* about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week** ⇨

2b. How much time in total did you usually spend on one of those days doing moderate physical activities*?*

_____ **hours** _____ **minutes**

or

☐ **none**

3a. During the last 7 days, on how many days did you **walk** for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

_____ **days per week** ⇨

3b. How much time in total did you usually spend walking on one of those days*?*

_____ **hours** _____ **minutes**

or

☐ **none**

The last question is about the time you spent sitting on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.

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

____ **hours** _____ **minutes**

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

This is the final SHORT LAST 7 DAYS SELF-ADMINISTERED version of IPAQ from the 2000/01 Reliability and Validity Study. Completed May 2001.

10.5 Appendix V: The physical activity readiness questionnaire

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	1. **Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2. **Do you feel pain in your chest when you do physical activity?**
☐	☐	3. **In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4. **Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5. **Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6. **Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7. **Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

CSEP | SCPE

10.6 Appendix VI: The 'RISK' scoring system

EUROPEAN SOCIETY OF CARDIOLOGY SYSTEMATIC CORONARY RISK EVALUATION (SCORE) AND MODIFIED RISK CRITERIA

Leicester-Loughborough
Diet, Lifestyle and Physical Activity
Biomedical Research Unit

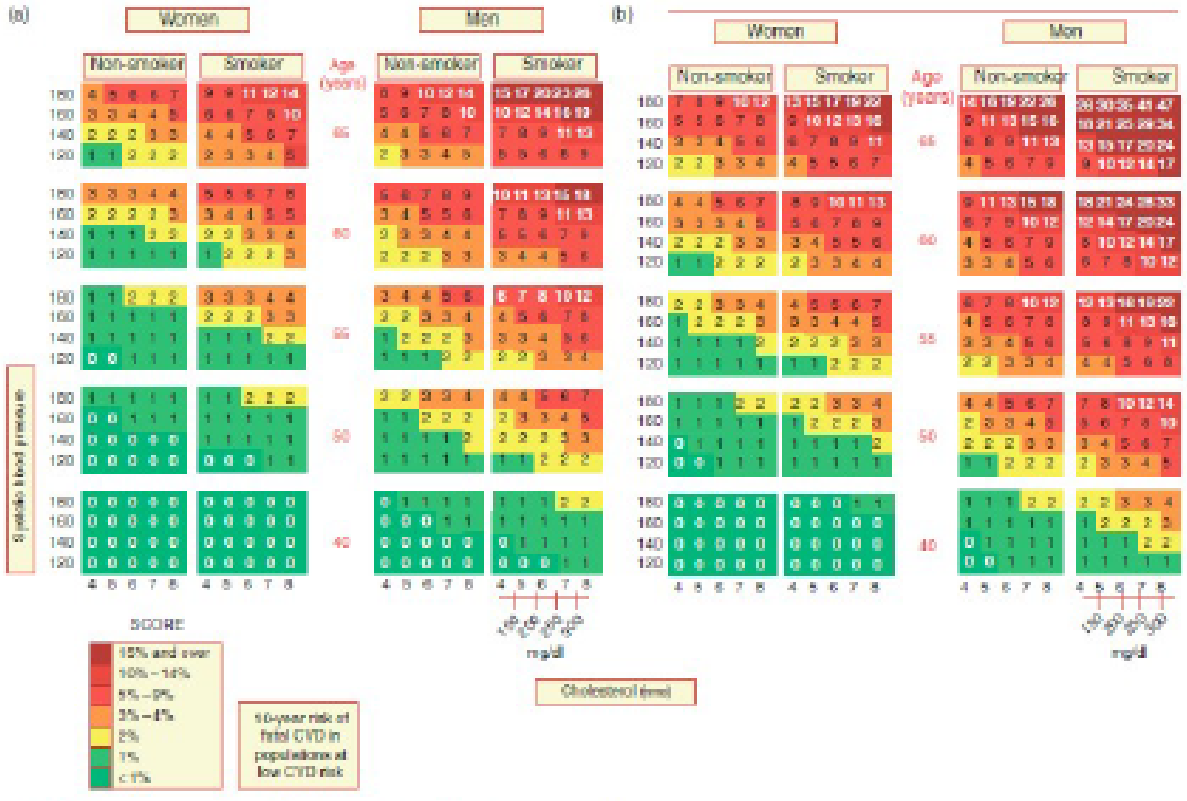
As part of the participant evaluation it is recommended that the SCORE system is used to estimate the absolute risk of atherosclerotic cardiovascular disease in the next 10 years. This algorithm is based on:

1. Age
2. Sex
3. Blood pressure
4. Cholesterol (total)
5. Smoking history

High risk is defined as possessing multiple cardiac risk factors resulting in a 10 year risk greater than 5%, or if extrapolated to 60 years in the SCORE chart.

The high risk factor profile for developing a fatal cardiovascular event is also defined as one of the following which represent exceptionally high individual risk factors:

- Previous history of, or diagnosed chronic disease
- Raised total cholesterol (> 8 mmol/L), LDL-C (> 6mmol/L)
- Raised blood pressure (180/110 mmHG)
- Individuals with a strong family history of CVD (myocardial infarction or stroke) in first degree relatives under 50 years of age
- Individuals with a BMI > 35 kg/m^2



SCORE risk in low (a) or high-risk (b) countries. CVD, cardiovascular disease.

10.7 Appendix VII: Supplementary results for Chapter 4

Table 10.1 Associations between fasted hepatokine concentrations and metabolic biomarkers.

		Age	*Body mass*	*BMI*	*BF%*	*WC*	*Relative $\dot{V}O_2$ peak*	*TG*	*NEFA*	*FPI*	*Fasted plasma glucagon*	*HOMA -IR*	*Adipo-IR*	*AST*	*ALT*	*AST: ALT*	*FGF21*
FGF21	*Corr.*		0.74	0.71	0.68	0.76	-0.52	0.72	0.79		0.44#		0.70#				
	P-value		<0.001	<0.001	0.001	<0.001	0.01	<0.001	<0.001		0.04		<0.001				
LECT2	*Corr.*		0.47*	0.49	0.60*	0.42*	-0.53	0.55#	0.52*	0.64#	0.49#	0.66#	0.70#	0.44#			0.61*
	P-value		0.03	0.02	<0.01	0.05	0.01	<0.01	0.01	0.001	0.02	0.001	<0.001	0.04			<0.01
Follistatin	*Corr.*															-0.43#	
	P-value															0.05	
Fetuin-A	*Corr.*	-0.50*															
	P-value	0.02															
SeP	*Corr.*														-0.44#		
	P-value														0.04		

Corr: correlation coefficient (*r* or *rho* for Pearson's and Spearman's analysis, respectively; Data are analysed using parametric Pearson's correlation analysis unless otherwise stated. * indicates non-parametric Spearman Rank correlation analysis used. # indicates data were log transformed prior to analysis.

10.8 Appendix VIII: Supplementary methods for Chapter 6

10.8.1 Self-report alcohol intake questionnaire

Loughborough University

Alcohol Intake Questionnaire:
Participant code: .....

Do you drink Alcohol? Yes No
(If yes, Please answer the questions below)

GUIDE TO ALCOHOL UNITS	 Pint of regular beer/lager/cider = 2 units	 Alcopop or can of lager = 1.5 units	 Glass of wine (175ml) = 2 units	 Single measure of spirits = 1 unit	 Bottle of wine = 9 units

Questions	Scoring System 0	1	2	3	4	Your Score
How often do you have a drink that contains alcohol?	Never	Monthly or less	2-4 times a month	2-3 times per week	4+ times per week	
How many standard alcohol units do you have on a typical day when you are drinking?	1-2	3-4	5-6	7-9	10+	
How often do you have 6 or more standard alcohol units on one occasion?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
If you score a total of 5 or more on the above questions, please complete the further questions below.						
How often in the last year have you found that you were not able to stop drinking once you have started?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
How often in the last year have you failed to do what was expected of you because of drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
How often in the last year have you needed an alcoholic drink in the morning to get you going?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
How often in the last year have you had a feeling of guilt or regret after drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
How often in the last year have you not been able to remember what happened when drinking the night before?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
Have you or someone else been injured as a result of your drinking?	No		Yes, but not in the last year		Yes, during the last year	
Has a relative/friend/doctor or health worker been concerned about your drinking or advised you to cut down?	No		Yes, but not in the last year		Yes, during the last year	
Your total score for all ten questions indicates the following: 0-7 = sensible drinking 8-15 = hazardous drinking 16-19 = harmful drinking 20+ = possible dependence						

This questionnaire is based the NHS alcohol self-assessment tool found online at:
http://www.nhs.uk/Tools/Pages/Alcoholcalculator.aspx

Loughborough Study 2014

Clamp Sheet

Subject ID : .....

Date : ...../...../..... **Visit No:** 1 2 3

Height: ..... cm **Weight:** ..... kg **Surface area:** .....m^2

Calculations:

15mls of stock solution are provided by pharmacy @ 200mg/ml

Tracer Bolus;
Take 5ml of stock solution (1000mg glucose) and make up to 50ml with 0.9% saline in a 50ml syringe to give a 20mg/ml solution

4mg x _____kg = _____mg / 20 = _____ml bolus delivered over 1min

Tracer Infusion;
Take 5ml of stock solution (1000mg) and make up to 50ml with 0.9% saline in a 50ml syringe

0.04mg x_____kg x 60min = _____/(20mg/ml) = _____ml/hr
6 hour Infusion @

Glucose Infusion;
Take 5ml of stock solution (1000mg) and add to a 500ml bag of 20% glucose to give ~1% enrichment

_____ m^2	x	20	=	x 7.52 = ... mls/hr
0.5		40		x 3.28 = ... mls/hr
				x 2.99 = ... mls/hr
				x 2.82 = ... mls/hr
				x 2.57 = ... mls/hr
				x 2.40 = ... mls/hr

_____ m^2	x	50	=	x 7.52 = ... mls/hr
0.5		40		x 3.28 = ... mls/hr
				x 2.99 = ... mls/hr
				x 2.82 = ... mls/hr
				x 2.57 = ... mls/hr
				x 2.40 = ... mls/hr

Glucose @ 2mg / kg / min @ 4 min

$\frac{2 \times \text{kg}}{200\text{mg}} \times 60 = \ldots\ldots\ldots\text{ml/hr}$

Glucose @ 2.5mg / kg / min @ 10 min

$\frac{2.5 \times \text{kg}}{200\text{mg}} \times 60 = \ldots\ldots\ldots\text{ml/hr}$

Infusion start time: .....

Time	Ins Inf^{n}	Blood G	G Inf^{n}	Ins / tracer sample	Calorimetry	Comments
-65				1		
-60				2		
-20				3		
-10						
-5						
0				4		
2						
4						
5						
6						
8						
10						
15				5		
20						
25						
30				6		
35						
40						
45				7		
50						
55						
60				8		
65						
70						
75				9		
80						
85						
90				10		
95						
100						
105				11		
110						
115						
120				12		
122						
124						
125						
126						
128						
130						
135				13		
140						
145						
150				14		

Time	Ins Inf^{n}	Blood G	G Inf^{n}	Ins / tracer sample	Calorimetry	Comments
155						
160						
165				15		
170						
175						
180				16		
185						
190						
195				17		
200						
205						
210				18		
215						
220						
225				19		
230						
235						
240				20		
	Subject	fed and	insulin	discontinued		
250						
260						
270						
280						
290						
300						
310						
320						
330						
340						
350						
360						

Comments:

10.8.2 Further details of accelerometry data collection and analysis

To assess the impact of SIT on habitual physical activity levels, participants wore a tri-axial accelerometer (GT3x, Actigraph LLC, USA) for seven consecutive days in the week before study assessments. Participants were encouraged to wear the device at all times, with the exception of showering/bathing or other water-based activities such as swimming. Participants were provided with a diary (see below) to note any points at which they removed the device, waking and sleeping times, physical activity performed and whether days were work or non-work days. Participants were free to remove the device overnight, but were asked to note this on the diary and to remove the device as late as possible and put it back on as soon as they woke up. Data processing methods have been described in-depth previously (Troiano *et al.*, 2008) and were facilitated using computer software (Kinesoft 3.3.80, USA). A period of monitoring was considered valid for analysis when data was available for at least 600 minutes of wear time on at least three days of the week. In all but one period of monitoring, data included at least one weekend day. Non-wear time was considered 60 minutes of consecutive zero counts, allowing for two minutes of interruption. The cut-points in counts per minute (CPM) that were used to determine different categories of activity are presented in Table 10.2.

Table 10.2 Troiano cut-points for categories of physical activity in counts per minute using tri-axial accelerometry analysis

	Sedentary Behaviour	Light Physical Activity	Moderate Physical Activity	Vigorous Physical Activity
Counts per minute	< 100	100 to 2019	2020 to 5999	> 5999

Participant ID: _____ Unit Serial No: _____

LIVE Study - How to wear the hip monitor

- The hip monitor or Actigraph measures the intensity of your physical activity (light, moderate or vigorous activity).
- It is to be worn **for 7 days**. However, it should be removed when bathing, showering and/or swimming. If you find it uncomfortable to sleep with, you can take it off but it is very important that you **put it on as soon as you wake up in the morning.**
- The monitor should be placed on, or as close to, your waistband as possible and rest on your hip bone, either side is OK.
- The monitor can be worn either underneath or on top of your clothing, just as long as it fits snugly around your waistband.
- The black button on top of the device should be facing up when being worn

How to fill in the daily log

- The log is divided into 7 days. Please complete each day's questions as accurately as possible – record the exact times or to the nearest 5 minutes.

1. Indicate the date.
2. Record the time that you **woke up** and when you put the waist device on.
3. State if it's a **work or non-work day**.
4. Indicate if you trained or not on that day
5. Indicate if you slept with the device on
6. Indicate the time you went to bed
7. Finally, say whether you took the device off at any point in the day and in the box to the right add detail about this

If you have any problems or concerns regarding this device don't hesitate to contact me.

Jack Sargeant – 07904 688 848
or alternatively:
James King – 07951 523 959

Participant ID: _____ Unit Serial No: _____

Date: ___/___/___ Waking up time?	What time did you put the waist device on?	Is today a work or non-work day?	Did you train today? If yes, what time?	Did you go to sleep with the waist device on?	At what time did you go to bed?	Did you remove the waist device at any time in the day?	Detail regarding device removal i.e. 11:30 for 20 mins
01/04/14 7:30 am/pm	7:35 am/pm	Work Non-work	e.g. 17:00___ am/pm	Yes / no	23:30 am / pm	20:20am/pm 20:50am/pm	
Day 1 ___/___/___ ____ am/pm	____ am/pm	Work Non-work	____ am/pm	Yes / no	____ am/pm	____ am/pm ____ am/pm	
Day 2 ___/___/___ ____ am/pm	____ am/pm	Work Non-work	____ am/pm	Yes / no	____ am/pm	____ am/pm ____ am/pm	
Day 3 ___/___/___ ____am/pm	____ am/pm	Work Non-work	____ am/pm	Yes / no	____ am/pm	____ am/pm ____ am/pm	

Participant ID: _____ Unit Serial No: _____

Day 4 ___/___/___ ____ am/pm	____ am/pm	Work Non-work	____ am/pm	Yes / no	____ am/pm	____ am/pm ____ am/pm	
Day 5 ___/___/___ ____ am/pm	____ am/pm	Work Non-work	____ am/pm	Yes / no	____ am/pm	____ am/pm ____ am/pm	
Day 6 ___/___/___ _____am/pm	____ am/pm	Work Non-work	____ am/pm	Yes / no	____ am/pm	____ am/pm ____ am/pm	
Day 7 ___/___/___ ____am/pm	____ am/pm	Work Non-work	____ am/pm	Yes / no	____ am/pm	____ am/pm ____ am/pm	

Participant ID: _____ Unit Serial No: _____

Do you have any comments about the daily log?

_____

_____

_____

_____

10.8.3 Full tracer calculations used in Chapter 6

10.8.3.1 Basal EGP

EGP was calculated using the following equations at both -20 and 0 min. The mean of the two values was then calculated and reported as basal EGP.

$$APE = (TTR_{measured} - TTR_{baseline}) / [1 + (TTR_{measured} - TTR_{baseline})] \times 100$$

Where APE = atoms percent excess, $TTR_{measured}$ = tracer to tracee ratio at timepoint of interest (-20 and 0 min samples) and $TTR_{baseline}$ = tracer to tracee ratio at baseline (prior to tracer infusion).

$$EGP\ (\mu mol \cdot kg^{-1} \cdot min^{-1}) = Ra = F / APE \times 100$$

Where F = the continuous tracer infusion rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$).

10.8.3.2 EGP at low-dose insulin infusion:

The following calculations were used to calculate EGP during low-dose insulin infusion:

$$Tracer\ Ra_{continuous} = \frac{\{F - pV \times [(C_1 + C_2) / 2] \times [(APE_2 - APE_1) / (t_2 - t_1)]\}}{[(APE_1 + APE_2) / 2]}$$

Where $Ra_{continuous}$ is the Ra contribution from the continuous tracer infusion ($\mu mol \cdot kg^{-1} \cdot min^{-1}$), pV is the fractional pool of distribution (a constant of $160 mL \cdot kg^{-1}$ was used in this study), C_1, C_2, APE_1, APE_2, t_1 and t_2 are glucose concentrations ($mmol \cdot L^{-1}$), enrichment (APE) and time (min) at consecutive timepoints of interest.

$$Tracer\ Ra_{variable} = TTR_{bag} \times GIR_2 / [(TTR_1 + TTR_2) / 2]$$

Where $Ra_{variable}$ is the Ra contribution from the tracer-spiked variable dextrose infusion ($\mu mol \cdot kg^{-1} \cdot min^{-1}$), TTR_{bag} is the assumed TTR within the dextrose bags, GIR_2 is the variable infusion rate at t_2 ($\mu mol \cdot kg^{-1} \cdot min^{-1}$), and TTR_1 and TTR_2 are the TTR and t_1 and t_2 respectively.

$$EGP\ (\mu mol \cdot kg^{-1} \cdot min^{-1}) = Ra_{continuous} + Ra_{variable} - GIR_2$$

10.9 Appendix IX: Supplementary results for Chapter 6

Table 10.3 Participant characteristics specific to those completing hyperinsulinaemic, euglycaemic clamps

Anthropometry	
Age (years)	40 ± 7
Body mass (kg)	102.1 ± 11.2
BMI (kg.m^{-2})	31.5 ± 3.3
Waist circumference (cm)	110.9 ± 8.0
Body fat (%)	28.1 ± 2.5
IHTG (%)	16.0 ± 8.8
Metabolic Risk Factors	
Alcohol Intake (units.week^{-1})$^{\#}$	4 (3 – 10)
Total Cholesterol (mmol.L^{-1})	4.74 ± 0.44
HDL (mmol.L^{-1})	0.98 ± 0.23
LDL (mmol.L^{-1})	2.70 ± 0.43
Cardiorespiratory Fitness	
Absolute $\dot{V}O_2$ peak (L.min^{-1})	3.24 ± 0.44
Relative $\dot{V}O_2$ peak (mL.kg^{-1}.min^{-1})	32.0 ± 5.1
Absolute PPO (W)	243 ± 44
Relative PPO (W.kg^{-1})	2.40 ± 0.52

Data presented as mean ± SD for *n*=8. $^{\#}$Data not normally distributed and thus presented as median (IQR).

Table 10.4 Participant characteristics specific to those for which full VAT and ScAT data are available

Anthropometry	
Age (years)	42 ± 7
Body mass (kg)	99.9 ± 7.7
BMI ($kg.m^{-2}$)	31.0 ± 2.5
Waist circumference (cm)	110.0 ± 6.3
Body fat (%)	28.4 ± 3.0
IHTG (%)	14.2 ± 7.7
Metabolic Risk Factors	
Alcohol Intake ($units.week^{-1}$)[#]	4 (3 – 10)
Total Cholesterol ($mmol.L^{-1}$)	4.88 ± 0.63
HDL ($mmol.L^{-1}$)	0.98 ± 0.26
LDL ($mmol.L^{-1}$)	2.83 ± 0.65
Cardiorespiratory Fitness	
Absolute $\dot{V}O_2$ peak ($L.min^{-1}$)	3.24 ± 0.46
Relative $\dot{V}O_2$ peak ($mL.kg^{-1}.min^{-1}$)	32.5 ± 4.6
Absolute PPO (W)	241 ± 45
Relative PPO ($W.kg^{-1}$)	2.42 ± 0.48

Data presented as mean ± SD for n=8. [#]Data not normally distributed and thus presented as median (IQR).

10.10 Appendix X: Supplementary methods for Chapter 7

10.10.1 Data extraction

10.10.1.1 Imputation of the mean and standard deviation (SD) during data extraction

When the variation of change from pre- to post-intervention was reported as standard error or confidence intervals, established statistical equations were used to convert these to SD. When only pre- and post-intervention data were available, the mean change and SD were imputed as previously reported (Higgins and Green, 2011), assuming a correlation coefficient for change in IHTG of 0.80 (Pugh *et al.*, 2014; Hallsworth *et al.*, 2015; Houghton *et al.*, 2017).

10.10.1.2 Data presented as median and interquartile range

When original data were reported as median and interquartile range, the median change was extracted and used in place of the mean, whilst the IQR was divided by 1.35 as an estimate SD (Higgins and Green, 2011). A sensitivity analysis removing these studies suggested that using data in this manner had no substantial impact on the results reported.

10.10.1.3 Converting data to a consistent unit of measurement

Data presented in this meta-analysis are, as much as possible, presented in a consistent format using similar units of measurement. When IHTG was reported as the ratio between intracellular lipid and water (lipid:water), this was converted to liver fat fraction using the following calculation:

(lipid:water) / [1+(lipid:water)] x 100

In some cases, data were converted during extraction using conversion factors as follows:

Insulin: 1 mU = 6 pmol

Glucose: 1 $mmol \cdot L^{-1}$ = 18 $mg \cdot dL^{-1}$

EGP: 1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ = 0.18 $mg \cdot kg^{-1} \cdot min^{-1}$

When data were also normalised, such as when presenting aerobic capacity in $ml \cdot kg^{-1} \cdot min^{-1}$ rather than $L \cdot min^{-1}$, the mean value for the whole study group at baseline was used.

10.10.1.4 Inclusion of subsets of participants

When a paper reported one of the primary outcomes of this review in a subset of individuals (or when only a subset of individuals met the inclusion criteria), only these individuals were included. If descriptive data specific to this subset were available, they were extracted. If not, the sample size was adjusted to represent the subset of individuals but the mean descriptive data for the whole study population was used. This applies to the following studies:

Cuthbertson *et al*., 2016 (Cuthbertson *et al.*, 2016): only 19 individuals (ex: 12; con: 7) underwent hyperinsulinaemic, euglycaemic clamp and were thus included in HISI and percentage suppression of EGP analyses.

Hickman *et al*., 2013 (Hickman *et al.*, 2013): only nine of the 13 patients in the exercise group underwent post-training liver biopsy and were thus included in IHTG analyses. Furthermore, complete data to allow calculation of HISI were only available for 11 patients.

Keating *et al*., 2015 (Keating *et al.*, 2015): three separate exercise groups completing different exercise interventions were included in this study. One of these groups was ineligible as the mean baseline IHTG was < 5.56%.

Langleite *et al.,* 2016 (Langleite *et al.*, 2016): only the dysglycaemic individuals had IHTG > 5.56% at baseline.

Meex *et al*., 2010 (Meex *et al.*, 2010): Raw data was collected for the calculation of HISI. One participant was removed from analysis because no basal EGP data was available.

Sargeant *et al*., 2018 (Sargeant, Bawden, *et al.*, 2018): only eight of the nine participants completed hyperinsulinaemic, euglycaemic clamps and so only these participants were included in HISI and percentage suppression of EGP analyses.

van der Heijden *et al*., 2010 (van der Heijden, Wang, Chu, Toffolo, *et al.*, 2010): only seven individuals had NAFLD (defined as liver fat > 5.6%) and so only this subset was included in the hepatic steatosis analysis. All participants were included in the HISI analysis as NAFLD diagnosis was not an inclusion criterion for this outcome.

10.10.1.5 Inclusion of studies with multiple follow-up measurements

One manuscript (Zhang *et al.*, 2016) reported data after six months and 12 months of intervention in two separate exercise groups. The interventions differed only during the first six months and, therefore, these data were extracted and used.

10.11 Appendix XI: Supplementary results for Chapter 7

Table 10.5 Participant characteristics and outcome measures of studies assessing changes in IHTG (continued overleaf)

Ref. *Study Design*	Intervention Overview	Participant Characteristics at Baseline							Relative Change in Body Weight (%)	Comments
		N (M/F)	Age (years)	BMI ($kg•m^{-2}$)	Body Fat (%)	Aerobic Capacity ($ml•kg^{-1}•min^{-1}$)	Baseline IHTG (%)	Clinical Conditions		
Cassidy *et al*. (2016) *RCT*	HIIT 3 times per week for 12 weeks.	Ex: 12 (10/2) Con: 11 (8/3)	61 ± 9 59 ± 9	31 ± 5 32 ± 6	35.4 (est.) 39.6 (est.)	21.8 ± 5.4 20.3 ± 6.1	6.9 ± 6.9 7.1 ± 6.8	Hepatic steatosis according to mean baseline measures. Diagnosed T2DM with stable control for ≥ 6 months.	-1.1[#] 1.1	*Reported medications*: Metformin (Ex: n=7, Con: n=7) Statins (Ex: n=7, Con: n=6) Antihypertensives (Ex: n=3, Con: n=5)
Cuthbertson *et al*. (2016) *RCT*	Aerobic 3 – 5 times per week for 12 weeks.	Ex: 30 (23/7) Con: 20 (16/4)	50 (46–58) 52 (46–59)	30.6 (29.0–32.9) 29.7 (28.0–33.0)	30.4 (25.9-32.1) 31.0 (26.5-37.7)	23.7 (21.7–27.8) 32.2 (20.9–25.6)	19.4 (14.6–36.1) 16.0 (9.6–32.5)	Diagnosed NAFLD	-2.5 (-3.5 – -1.4)[α,#] 0.2 (-0.8–1.1)	*Notable exclusion criteria*: T2DM and IHD Excessive weight loss during the course of the study
Hallsworth *et al*. (2011) *RCT*	Resistance 3 times per weeks for 8 weeks.	Ex: 11 (NR) Con: 8 (NR)	52 ± 13 62 ± 7	32.3 ± 4.9 32.3 ± 4.8	37 ± 8 41 ± 6	21.8 ± 3.8 18.5 ± 5.2	14.0 ± 9.1 11.2 ± 8.4	Clinically defined but non-advanced NAFLD (defined as IHTG > 5% with NAFLD fibrosis score < -1.445). T2DM accepted provided diet and metformin prescription were stable for ≥ 6 months.	0.0 0.6	*Notable exclusion criteria*: Exogenous insulin therapy. IHD Weight loss > 2.5% during the study. *Other:* Exercise group were significantly younger than control ($p < 0.05$).
Hallsworth *et al*. (2015) *RCT*	HIIT 3 times per week for 12 weeks.	Ex: 12 (6/6) Con: 11 (10/1)	54 ± 10 52 ± 12	31.0 ± 4.0 31.0 ± 5.0	38.4 ± 6.4 34.5 ± 7.0	21.9 ± 6.2 24.6 ± 5.7	10.6 ± 4.9 10.3 ± 4.4	Clinically defined but non-advanced NAFLD (defined as IHTG > 5% with NAFLD fibrosis score < -1.445). Metformin accepted but participants with any other medication for T2DM were excluded.	-1.6[α,#] 0.0	None
Haus *et al*. (2013) *Uncontrolled Intervention*	Aerobic Daily for 1 week.	Ex: 17 (NR)	54 ± 2	34.4 ± 1.0	NR	24.3 ± 1.5	19.4 ± 3.3	Diagnosed NAFLD.	0.2	*Notable exclusion criteria*: T1DM,T2DM and CVD

Hickman *et al*. (2013) *RCT*[*]	Resistance 3 times per week for 24 weeks.	Ex: 9 (7/2)	48 ± 9	33.6 ± 5.8	38.8 ± 7.6	21.6 ± 7.3	71 ± 32[†]	Diagnosed NAFLD. 85% met diagnostic criteria for NASH.	-2.6 ± 4.65	*Notable exclusion criteria*: Diabetes Alcohol consumption > 40 and 20 g•day^{-1} for men and women respectively.
Houghton *et al*. (2017) *RCT*	Combined (HIIT + Resistance) 3 times per week for 12 weeks.	Ex: 12 (7/5) Con: 12 (7/5)	54 ± 12 51 ± 16	33.0 ± 7.0 33.0 ± 5.0	NR	25.0 ± 8.0 21.0 ± 5.0	12.0 ± 9.0 10.0 ± 5.0	Histologically characterised NASH with NAFLD activity score ≥ 5.	1.1 1.1	*Notable exclusion criteria*: ≥60 minutes moderate-vigorous physical activity per week Insulin sensitising treatments Cardiac or renal diseases.
Johnson *et al*. (2009) *RCT*	Aerobic 3 times per week for 4 weeks.	Ex: 12 (NR) Con: 7 (NR)	49 ± 8 47 ± 10	32.2 ± 2.8 31.1 ± 2.9	NR	25.9 ± 4.8 25.0 ± 4.2	8.6 ± 8.6 9.2 ± 10.1	Hepatic steatosis according to mean baseline measures. Hypertension allowed.	-0.3 -0.2	*Notable exclusion criteria*: Lipid lowering medications FPG ≥ 7.0 mmol•L^{-1} *Reported medications*: Anti-hypertensive medications (n=5) Note – medications were unaltered for the duration of the study except when participants refrained for 72 hours prior to study assessments.
Keating *et al*. (2015) *RCT*	Aerobic 3-4 times per week for 8 weeks.	Ex1(HI:LO): 12 (6/6) Ex2(LO:HI): 11 (5/6) Con: 12 (3/9)	44 ± 10 45 ± 9 39 ± 10	36.3 ± 5.9 34.0 ± 3.1 32.2 ± 4.8	NR	21.9 ± 4.8 24.5 ± 3.0 21.7 ± 6.2	8.4 ± 5.2 9.4 ± 6.6 7.7 ± 9.0	Hepatic steatosis according to mean baseline measures.	-1.2[α,#] -1.5[α,#] 0.9[a]	*Notable exclusion criteria*: Reported exercise on >3 days per week. Lipid-lowering or insulin-sensitizing medications.
Langleite *et al*. (2016) *Uncontrolled Intervention*	Combined (Aerobic + HIIT + Resistance) 4 times per week for 12 weeks	Ex: 11 (11/0)	53 (10)	27.8 (5.3)	NR	38.7 (8.1)	11.0 (11.9)	Hepatic steatosis according to mean baseline measures Dysglycaemic according to OGTT performed during screening.	-1.2	*Notable exclusion criteria*: Structured exercise performed > once per week Hypertension, other liver or kidney diseases, chronic inflammatory disease or medications known to affect glucose metabolism.
Malin *et al*. (2013) *Uncontrolled Intervention*	Aerobic Daily for 1 week.	Ex: 13 (6/7)	51 ± 12	33.3 ± 3.2	NR	24.9 ± 5.4	23.1 ± 14.8	Hepatic steatosis confirmed during baseline measures (defined as > 5% IHTG).	0.6	None

Oh *et al*. (2014) *Uncontrolled Intervention*	Acceleration / Vibration 3 times per week for 6 weeks.	Ex: 18 (4/14)	NR	28.2 (25.5–33.2)	NR	NR	12.2 (5.4–20.6)	Diagnosed NAFLD by medical history, serum ALT and ultrasound. Confirmed by baseline IHTG > 5%.	-0.4[a]	None
Pugh *et al*. (2014) *RCT*	Aerobic 3 – 5 times per week for 16 weeks.	Ex: 13 (7/6) Con: 8 (4/4)	50 ± 3 47 ± 5	30.0 ± 0.8 30.0 ± 2.0	NR	26.4 ± 2.3 27.0 ± 2.8	21.3 ± 12.8 19.2 ± 6.1	Clinically diagnosed NAFLD defined as IHTG ≥ 5.5% Individuals taking anti-hypertensive medications were allowed.	-2.4 ± 2.0 -1.1 ± 2.0	*Notable exclusion criteria*: Any form of structured exercise or > 2 hours of low-intensity physical activity per week. T2DM, IHD, habitual smokers. *Reported medications*: Anti-hypertensive medications (Ex: n=9) Note - medications were unaltered throughout the duration of the study.
Sargeant *et al*. (2018) *Controlled Longitudinal Intervention*	HIIT 3 times per week for 6 weeks.	Ex: 9 (9/0)	41 ± 8	31.7 ± 3.1	28.7 ± 3.0	31.8 ± 4.8	15.6 ± 8.3	NAFLD defined as IHTG ≥ 5.56 percent in the absence of reported secondary causes as determined during participant screening.	-1.2	Participants were weight stable *Notable exclusion criteria:* Any form of diagnosed metabolic disease or taking medication known to influence lipid metabolism or glycaemic control.
Sullivan *et al*. (2012) *RCT*	Aerobic 5 times per week for 16 weeks.	Ex: 12 (4/8) Con: 6 (1/5)	49 ± 8 48 ± 8	37.1 ± 3.8 40.0 ± 5.4	38.9 ± 2.1 42.5 ± 3.6	22.8 ± 4.5 18.5 ± 7.1	20.2 ± 14.6 21.4 ± 21.6	NAFLD defined as IHTG > 10%.	-0.2[a] 0.2[a]	*Notable exclusion criteria*: T2DM or plasma TG > 400mg•dL^{-1}. Not weight-stable (> 3kg weight change in previous three months). Self-reported exercise > 1 hour per week.
van der Heijden *et al*. (2010a)[‡] *Uncontrolled Intervention*	Aerobic 4 times per week for 12 weeks.	Ex: 15 (7/8)	16 ± 2	33.7 ± 4.3	38.3 ± 5.8	26.8 ± 6.3	9.0 ± 12.0	Hepatic steatosis according to mean baseline data.	-0.5	All participants were post-pubertal. Participants were obese for ≥5 years and weight stable for ≥ 6 months. *Notable exclusion criteria*: Participation in organised school athletic programme or ≥ 45 minutes self-reported light to moderate physical activity. Any form of metabolic disease. Any medication (including contraceptives). 1st degree relatives with diabetes. Morbid obesity (>50% body fat).

van der Heijden *et al*. (2010b) *Uncontrolled Intervention*	Resistance 2 times per week for 12 weeks.	Ex: 7 (NR)	16 ± 1	35.3 ± 1.9	42.6 ± 5.3	NR	13.9 ± 11.4	Hepatic steatosis according to mean baseline measures but no formal diagnosis of NAFLD reported.	2.6$^{\alpha}$	All participants were post-pubertal. Participants were obese for ≥5 years and weight stable for ≥6 months. *Notable exclusion criteria*: Participation in any organised school athletic programme or ≥ 45 minutes self-reported light to moderate physical activity. Any form of metabolic disease. Any medication (including contraceptives). 1st degree relatives with diabetes. Morbid obesity (>50% body fat).
Zhang *et al* (2016) *RCT*	Aerobic 5 times per week for 24 weeks.	Ex1(Mod): 73 (22/51) Ex2(Vig): 73 (21/52) Con: 74 (28/46)	54 ± 7 53 ± 7 54 ± 7	28.1 ± 3.3 27.9 ± 2.7 28.0 ± 2.7	33.5 ± 5.5 34.8 ± 5.3 33.7 ± 7.1	NR	18.0 ± 9.9 18.4 ± 9.9 17.5 ± 11.0	NAFLD diagnosed initially by ultrasound and the confirmed by ^{1}H-MRS during screening	-2.8 $^{\alpha, \#}$ -6.0 $^{\alpha, \#}$ -2.1 $^{\alpha,}$	*Notable exclusion criteria*: History of other chronic liver diseases, hypertension, chronic kidney disease, hyperthyroidism, myocardial infarction (within 6 month) or heart failure. Participation in weight loss programmes

Data presented as mean ± SD or median (IQR); Sample sizes represent the number of individuals entered into analyses; *Study did not include 'standard care' or 'no intervention' group. Patients were randomised to either exercise or dietary interventions; † Liver biopsy used. This number represent the percentage of hepatocytes affected by steatosis; ‡ Manuscript refers to same study as van der Heijden *et al* (2009); $^{\alpha}$ significant difference from baseline ($P < 0.05$); $^{\#}$ significant interaction between exercise and control groups ($P < 0.05$).

Table 10.6 Participant characteristics and outcome measures of studies assessing changes in HISI and %EGP_{supp} (continued overleaf)

Ref. *Study Design*	Intervention Overview	Participant Characteristics at Baseline										Comments
		N (M:F)	Age (years)	BMI ($kg•m^{-2}$)	Body Fat (%)	Aerobic Capacity ($ml•kg^{-1}•min^{-1}$)	Fasted Glucose ($mmol•L^{-1}$)	Fasted Insulin ($\mu U•mL^{-1}$)	HISI ($mg•m^{-2}•min^{-1}$ per $\mu U•mL^{-1}$)	EGP suppression (%)	Clinical Conditions	
Cuthbertson *et al*. (2016) *RCT*	Aerobic 3 – 5 times per week for 12 weeks.	Ex: 12 (8/3/1NR) Con: 7 (3/4)	44 ± 13 50 ± 12	31.0 ± 2.3 29.0 ± 3.2	30.4 (25.9-32.1) 31.0 (26.5-37.7)	28.1 ± 7.2 24.2 ± 11.3	4.79 ± 0.42 4.84 ± 0.64	17.4 ± 11.1 13.4 ± 4.9	1.11 ± 0.60 1.00 ± 0.58	50.1 ± 20.2 46.5 ± 27.3	Diagnosed NAFLD.	*See Table 4.2 for Notable exclusion criteria* *Insulin Dose and Infusion Duration:* 0.3 $mU•kg^{-1}•min^{-1}$ for 120 minutes. *Potential Confounding Variables:* Significant reduction in body weight and IHTG in the exercise group.
Hickman *et al*. (2013) *RCT*[*]	Resistance 3 times per week for 24 weeks.	Ex: 13 (9/4)	50 ± 9	33 ± 6	39 ± 8	NR	5.5 ± 0.5	24 ± 22	0.85 ± 0.61	Not Measured	Diagnosed NAFLD. 85% met diagnostic criteria for NASH	*See Table 4.2 for Notable exclusion criteria* NOTE: $\%EGP_{supp}$ was reported at high-dose insulin infusion (1 $mU•kg^{-1}•min^{-1}$) and was improved by exercise training.
Lee *et al*. (2013) *RCT*	Ex 1: Aerobic Ex 2: Resistance 3-4 times per week for 8 weeks.	Ex1: 16 (0/16) Ex2: 16 (0/16) Con: 12 (0/12)	15 ± 2 15 ± 2 15 ± 2	32.9 ± 3.8 36.4 ± 3.8 35.3 ± 4.0	47.8 ± 4.2 51.5 ± 4.7 51.3 ± 3.5	28.5 ± 3.8 24.3 ± 4.3 23.9 ± 3.0	5.18 ± 0.33 5.24 ± 0.38 5.39 ± 0.32	28.6 ± 16.5 45.8 ± 22.0 31.1 ± 15.3	24.2 ± 12.2[†] 16.5 ± 10.5[†] 22.9 ± 14.4[†]	Not Measured	None reported.	*Notable exclusion criteria*: Endocrine disorders (PCOS, T2DM) Medication known to influence glucose metabolism or body composition. *Potential Confounding Variables:* No change in body weight in either group. IHTG significantly reduced in aerobic exercise group only.

Meex *et al*. (2010) *Uncontrolled Intervention*	Combined (Aerobic + Resistance) 3 times per week (2 Aerobic, 1 Resistance) for 12 weeks.	Ex1: 20 (20/0) Ex2: 17 (17/0)	59 ± 1 59 ± 1	29.7 ± 3.6 30.0 ± 3.4	31.5 ± 1.4 31.1 ± 1.4	28.8 ± 4.5 27.5 ± 5.1	5.90 ± 0.45 9.00 ± 1.70	18.1 ± 10.7 17.0 ± 5.3	1.1 ± 0.5 0.8 ± 0.3	Not Measured	Ex1: None reported. Ex2: T2DM diagnosed for at least 1 year prior to study participation	*Notable exclusion criteria*: Cardiac disease, impaired liver or renal function, BMI > 35kg•m^{-2}. Exogenous insulin therapies. *Medications:* All T2DM participants were on oral antidiabetic agents. Medication was unchanged throughout the duration of the study but discontinued for 7 days prior to each clamp assessment. *Potential Confounding Variables:* No change in body weight in either group. IHTG not measured.
Sargeant *et al*. (2018) *Controlled Longitudinal Intervention*	HIIT 3 times per week for 6 weeks.	Ex: 8 (8/0)	40 ± 7	31.5 ± 3.3	28.1 ± 2.5	32.0 ± 5.1	4.7 ± 0.3	17.6 ± 4.5	0.68 ± 0.20	59.9 ± 17.4	NAFLD defined as IHTG ≥ 5.56 percent in the absence of reported secondary causes as determined during participant screening.	*See Table 4.2 for Notable exclusion criteria* *Insulin Dose and Infusion Duration:* 20 mU•m^{-2}•min^{-1} for 120 minutes. *Potential Confounding Variables:* No change in body weight. IHTG significantly reduced from pre- to post-training.

Shojaee-Moradie *et al*. (2007) *RCT*	Aerobic 3 times per week for 6 weeks.	Ex: 10 (10/0) Con: 7 (7/0)	47 ± 9 55 ± 11	27.6 ± 1.9 27.6 ± 2.4	25.6 (est.) 24.4 (est.)	31.0 ± 3.2 27.0 ± 5.3	NR NR	10.6 ± 6.3 11.4 ± 7.6	2.09 ± 1.03 1.91 ± 0.87	57.0 ± 15.1 62.0 ± 18.9	None reported.	*Notable exclusion criteria*: T2DM, hyperlipidaemia Lipid-lowering medications Those already engaged in regular physical activity. *Insulin Dose and Infusion Duration:* 0.3 mU•kg^{-1}•min^{-1} for 120 minutes. *Potential Confounding Variables:* No change in either body weight or IHTG in either group.
van der Heijden *et al*. (2009)‡ *Uncontrolled Intervention*	Aerobic 4 times per week for 12 weeks.	Ex: 15 (7/8)	16 ± 2	33.2 ± 3.5	38.3 ± 5.8	27.5 ± 6.3	5.0 ± 0.4	20.2 ± 9.6	0.87 ± 0.43	Not Measured	Hepatic steatosis according to mean baseline measures.	*See Table 4.2 for Notable exclusion criteria* *Potential Confounding Variables:* No change in body weight. IHTG not measured.
van der Heijden *et al*. (2010b) *Uncontrolled Intervention*	Resistance 2 times per week for 12 weeks.	Ex: 12 (6/6)	16 ± 2	35.3 ± 2.4	42.6 ± 5.3	NR	5.10 ± 0.35	23.0 ± 6.4	0.63 ± 0.18	Not Measured	Hepatic steatosis according to mean baseline measures.	*See Table 4.2 for Notable exclusion criteria* *Standardisation:* Post-intervention assessments were performed 3 days after the final exercise training session. *Potential Confounding Variables:* Body weight significantly increased from baseline. Much of this was accounted for by an increase in LBM. IHTG not measured.

Data presented as mean ± SD or median (IQR); Samples sizes represent the number of individuals entered into analyses; * Study did not include 'standard care' or 'no intervention' group. Patients were randomised to either exercise or dietary interventions; † HISI presented as mg•kg^{-1}•min^{-1} per μU•mL^{-1}; ‡ Manuscript refers to same study as van der Heijden *et al* (2010a); # baseline values not reported and unavailable upon request.

Table 10.7 Intervention characteristics of all included studies (continued overleaf)

Ref. *Study Design*	Exercise Type	Intervention Duration	Session Frequency	Details of Exercise intervention	Exercise Supervision	Instructions to Control Groups / Details of Placebo Intervention	Other Instructions
Cassidy *et al*. (2016) *RCT*	HIIT	12 weeks	3 times per week	Sessions consisted of 5 cycling intervals at an intensity equating to 'very hard' (16-17) on a Borg RPE scale. Interval length progressed by 10 seconds per week from 2 minutes in week 1 to 3 minutes 50 seconds by week 12. Intervals were interspersed with 3 minutes consisting of 90 seconds passive recovery, 60 seconds upper body resistance band exercise and 30 seconds preparation for the subsequent interval.	The initial exercise session was supervised by a member of the research team after which instructions were provided via voice-recordings loaded onto an iPod (Apple, CA, USA). Adherence was monitored via exercise diaries.	Continued standard care.	Participants were instructed to continue their normal routine and medical care, making no changes to diet, habitual activity or medication.
Cuthbertson *et al*. (2016) *RCT*	Aerobic	12 weeks	3 – 5 times per week	Participants were given the choice of exercising on a treadmill, cross-trainer, cycle ergometer or rower. Intensity increased from 30% HRR at week 1 to 60% HRR by week 12. Frequency and duration progressed from 30 minutes, 3 times per week during week 1 to 45 minutes, 5 times per week by week 12.	One session per week was supervised by a trained exercise physiologist. The remaining sessions monitored via Wellness System™ (Technogym U.K. Ltd.) or by repeated telephone/email contact.	Education and advice about the health benefits of exercise in NAFLD.	Participants in the exercise groups were instructed to make no dietary modifications (confirmed by 3-day self-report food diaries) To avoid disturbance to behaviour, participants in the control group were given no instructions regarding diet or lifestyle.
Hallsworth *et al*. (2011) *RCT*	Resistance	8 weeks	3 times per week	Sessions consisted of 8 whole-body exercises targeting large muscle groups performed as a circuit. Sessions progressed from 2 circuits at 50% 1RM during week 1 to 3 circuits at 70% 1RM by week 7. Participants were encouraged to increase the resistance each week if possible.	Sessions were supervised biweekly. Heart rate was recorded during every session and exercise logs were completed to monitor adherence.	Continued standard care.	None reported
Hallsworth *et al*. (2015) *RCT*	HIIT	12 weeks	3 times per week	Sessions consisted of 5 intervals on a cycle ergometer at an intensity equating to 'very hard' (16-17) on a Borg RPE scale. Interval length progressed by 10 seconds per week from 2 minutes in week 1 to 3 minutes 50 seconds by week 12. Intervals were interspersed with 3 minutes consisting of 90 seconds passive recovery, 60 seconds upper body resistance band exercise and 30 seconds preparation for the subsequent interval.	The first 2 exercise sessions were supervised by members of the research team after which instructions were provided via voice-recordings loaded onto an iPod (Apple, CA, USA). Exercise diaries were completed and reported completion of 33 out of 36 prescribed sessions was considered 'adequate adherence'.	Continued standard care.	Participants were instructed to continue their normal routine and medical care, making no changes to diet, habitual activity or medication. Participants were asked to monitor and maintain body weight within 1% of baseline.

Haus *et al*. (2013) *Uncontrolled Intervention*	Aerobic	1 week	7 consecutive days	Sessions lasted 50-60 minutes consisting of 40-50 minutes of walking or running on a treadmill at 80-85% HR_{max} with appropriate warm-up and cool-down.	All exercise sessions were supervised by an exercise physiologist.	N/A	Participants were instructed to maintain normal dietary habits and habitual physical activity.
Hickman *et al*. (2013) *RCT**	Resistance	24 weeks	3 times per week	Circuit-based sessions consisting of 15 moderate-intensity (50% 1RM) resistance exercise covering the main muscle groups. Each exercise was performed for 30 seconds with 30 seconds rest, during which participants moved to the next exercise. Sessions consisted of 1 circuit (12 min) during week 1 and progressed to 5 circuits (60 min) by week 12. 1RM was re-assessed every 4 weeks.	All exercise sessions were supervised	NA	There were no prescribed dietary changes for the exercise intervention group.
Houghton *et al*. (2017) *RCT*	Combined (HIIT + Resistance)	12 weeks	3 times per week	A short HIIT session was performed made up of a 5 minute warm-up followed by 3 x 2 minute intervals at an intensity equating to 'very hard' (16-18) on a Borg RPE scale. Intervals were interspersed with 1 minute rest. This was immediately followed by a resistance exercise circuit that comprised of 5 whole-body exercise targeting large muscle groups. Participants lifted a weight that equated to an RPE of 14-16 ('hard').	All exercise sessions were supervised by an accredited exercise specialist and recorded to ensure adherence.	Continued standard care with maintenance of baseline weight.	Participants were instructed to maintain normal dietary habits and habitual physical activity.
Johnson *et al*. (2009) *RCT*	Aerobic	4 weeks	3 times per week	Each session lasted 30-45 minutes consisting of 15 minute bouts of cycling with 5 minute recovery periods. Intensity was increased from 50% of pre-training $\dot{V}O_2$ peak during week 1 to 60% in week 2 and 70% in weeks 3 and 4.	All exercise sessions were supervised	30 minute home-based whole-body stretching routine.	Participants were instructed to maintain habitual diet throughout the study. 24 hour food records were collected on the first and final three training sessions.

Keating *et al*. (2015) *RCT*	Aerobic	8 weeks	Ex1 (HI:LO): 3 sessions per week Ex2 (LO:HI): 4 sessions per week	Ex1 (HI:LO): 2 laboratory-based cycling sessions and 1 home-based brisk walking session per week all at the same intensity and duration. The programme progressed as follows: Week 1: 45 minutes at 50% $\dot{V}O_2$ peak. Week 2: Individual progression. Weeks 3-8: 60 minutes at 50% $\dot{V}O_2$ peak. Ex2 (LO:HI): 3 laboratory-based cycling sessions and 1 home-based brisk walking session per week all at the same intensity and duration. The programme progressed as follows: Week 1: 30 minutes at 50% $\dot{V}O_2$ peak. Week 2: Individual progression. Weeks 3-8: 45 minutes at 70% $\dot{V}O_2$ peak.	All laboratory exercise sessions were supervised by an accredited exercise physiologist. Adherence was 90, 96 and 94% in the HI:LO, LO:HI and control groups respectively.	Stretching, self-massage and 'fitball' programme. 1 session per week were performed in the laboratory and the remaining 2 at home. During the one supervised laboratory session participants performed 5 min of cycling 30 W to maintain ergometer familiarity.	Participants were instructed to maintain habitual diet throughout the study. Participants completed 24 hour food records on 3 non-exercise days at baseline and during the final week of the intervention Participants also wore a tri-axial accelerometer for 2 weeks before and after the intervention.
Langleite *et al*. (2016) *Uncontrolled Intervention*	Combined (Aerobic + HIIT + Resistance)	12 weeks	3 times per week	Participants completed two whole-body strength training sessions and two sessions on a spinning bike per week. One bike session consisted of aerobic intervals for seven minutes at 85% HR_{max} with three minutes rest of active recovery against a light load between intervals. Participants completed three intervals in week one, four in weeks two-to-five and five from week six onwards. The second session consisted of two minute intervals at > 90% HR_{max} with two minutes rest of active recovery against a light load between intervals. Participants completed six intervals in week one, seven in weeks two-to-five and ten from week six onwards.	All exercise sessions were supervised Mean attendance was 90%.	N/A	Participants recorded habitual diet before and after the intervention.
Lee *et al*. (2013) *RCT*	Ex1: Aerobic Ex2: Resistance	13 weeks	3 times per week	Ex1: Aerobic exercise were performed on treadmill and/or elliptical. The programme progressed as follows: Week 1: 40 minutes at HR equating to ~50% $\dot{V}O_2$ peak. Weeks 2-8: 60 minutes at HR equating to ~70% $\dot{V}O_2$ peak. Ex2: 10 whole-body exercises targeting large muscle groups. All sessions were 60 minutes in duration. The programme progressed as follows: Weeks 1-4: 1-2 sets of 8-10 reps at 60% 1RM. Weeks 4-13: 2 sets of 8-12 reps to fatigue.	All exercise sessions were supervised by exercise physiology graduates. 4 participants did not complete exercise training (2 from each group). Mean (± SD) attendance was 95% (± 4.3%) and 97% (± 2.8%) in aerobic and resistance groups respectively.	Asked not to participate in any structured exercise activity To aid adherence, participants in control group were offered the opportunity to complete either exercise intervention following post-study assessment.	Participants consumed a weight-maintenance diet throughout the duration of the study.

Malin *et al*. (2013) *Uncontrolled Intervention*	Aerobic	1 week	7 consecutive days	Sessions lasted for approximately 60 minutes consisting of treadmill running at 85% HR_{max}.	All exercise sessions were supervised and 100% adherence was reported.	N/A	Participants were instructed to maintain normal dietary habits and habitual physical activity.
Meex *et al*. (2010) *Uncontrolled Intervention*	Combined (Moderate Aerobic + Resistance)	12 weeks	3 times per week total (2 aerobic, 1 resistance)	Aerobic: 30 minutes at 55% W_{max}. Resistance: 8 whole-body exercises targeting large muscle groups with 2 sets of 8 reps at 55% MVC.	Training sessions were supervised with 4 participants exercising per session.	N/A	None reported
Oh *et al*. (2014) *Uncontrolled Intervention*	Vibration / Acceleration	6 weeks	3 times per week	Whole-body exercises were performed using a vertical vibration machine. Sessions lasted 40 minutes with 30 seconds in between each exercise. Each week, one 'movement preparation', one 'strength and power' and one 'massage' session were performed.	Trained staff supervised all exercise sessions to ensure correct execution.	N/A	Participants received lifestyle counselling regarding diet and physical activity for NAFLD for 12 weeks prior to the intervention. This ceased at the beginning of the intervention. Participants completed 24 hour food records for 3 consecutive days and wore a uniaxial accelerometer for 2 weeks at baseline and during the final week of the intervention.
Pugh *et al*. (2014) *RCT*	Aerobic	16 weeks	3 – 5 times per week	Sessions consisted of a combination of treadmill and cycling exercise and progressed as follows: Weeks 1-4: 30 minutes at 30% HRR, 3 times per week. Weeks 4-8: 30 minutes at 45% HRR, 3 times per week. Weeks 8-12: 45 minutes at 45% HRR, 3 times per week. Weeks 12-16: 45 minutes at 60% HRR, 5 times per week.	Weekly sessions were supervised by a trained exercise physiologist.	Conventional care consisting of lifestyle advice from a consultant hepatologist or specialist nurse provided at a clinical consultation. No supervision or guidance was provided beyond this initial visit.	Participants in the exercise group were instructed to make no dietary modifications throughout the duration of the study, confirmed by 24 hour food diaries completed for 3 days before and after the intervention.
Sargeant *et al*. (2018) *Controlled Longitudinal Intervention*	HIIT	6 weeks	3 times per week	Sessions consisted of 30-second maximal sprints on cycle ergometer interspersed with 4.5-minute periods of active recovery at 50W. In addition participants completed a five-minute warm up and three-minute cool-down at 50W. Participants completed four intervals per session for the first two weeks after which an additional interval was added every two weeks, such that six intervals were completed per session in weeks five and six.	All exercise sessions were supervised and session attendance was 100%.	Each participant completed a six-week 'control phase' before the start of the training intervention, before and after which all study were assessed.	Participants were instructed to maintain habitual diet and lifestyle throughout the duration of the study.

Shojaee-Moradie *et al*. (2007) *RCT*	Aerobic (Vigorous)	6 weeks	3 times per week	Exercise performed at 60-85% $\dot{V}O_2$ max for a minimum of 20 minutes,	1 session per week was supervised by an exercise physiologist.	Participants were asked to continue their normal diet and lifestyle habits.	Participants were instructed not to change dietary habits throughout the study.
Sullivan *et al*. (2012) *RCT*	Aerobic	16 weeks	5 times per week	Sessions consisted of treadmill walking. During weeks 1-4, participants exercised for 15-30 minutes at 45-55% of pre-training $\dot{V}O_2$ peak. Sessions progressed regularly until participants performed 30-60 minutes of exercise at 45-55% $\dot{V}O_2$ peak and this was maintained for the remainder of the intervention.	1 session per week was performed under direct supervision from a member of the research team. The remaining sessions were completed at home.	Participants were instructed to maintain current activities of daily living and were contacted once per week to review compliance.	None reported
van der Heijden *et al*. (2010a)‡ *Uncontrolled Intervention*	Aerobic	12 weeks	4 times per week	Sessions consisted of treadmill, elliptical or cycle ergometer exercise. Sessions lasted approximately 50 minutes consisting of 10 minutes warm-up, 10 minutes cool down and 30 minutes exercise at a heart rate corresponding to 70% of baseline $\dot{V}O_2$ peak (mean ± SE: 86 ± 2% HR_{max}).	2 exercise sessions per week were supervised by an exercise physiologist and the remaining were performed at home with adherence monitored by recording of heart rate. On average, participants completed 91 ± 2% of prescribed sessions.	N/A	Participants were instructed to make no changes to dietary or physical activity habits during the duration of the study. Body weight was monitored two times per week to assure weight stability.
van der Heijden *et al*. (2010b) *Uncontrolled Intervention*	Resistance	12 weeks	2 times per week	Sessions lasted 60 minutes and consisted of 10 whole-body resistance exercises targeting large muscle groups. The programme progressed as follows: Weeks 1-2: 2-3 sets of 8-12 reps at 50% of 3RM. Weeks 3-8: individual progression increasing firstly by number of reps followed by weight. Weeks 9-12: 3 sets of 15-20 reps at 80-85% 3RM.	All exercise sessions per week were supervised by an exercise physiologist. On average, participants completed 96 ± 1% of the prescribed sessions.	N/A	Participants were instructed to make no changes to dietary or physical activity habits during the duration of the study. Body weight was monitored two times per week to assure weight stability.

Zhang *et al*. (2016) *RCT*	Aerobic	24 weeks	5 times per week	Sessions lasted 30 minutes. In the moderate exercise group, participants walked briskly at approximately 120 steps per minutes so that their heart rate was 45 to 55% of predicted $HR_{max.}$ In the vigorous exercise group, participants jogged at an intensity that elicited 65 to 80% of their predicted $HR_{max.}$	Participants were supervised during the first two to four weeks of training to familiarise themselves with the correct exercise intensity. Participant also attended bi-weekly health education sessions. Participants then performed sessions at a local community centre and received twice-weekly telephone calls to assess adherence. Participants in the moderate group were given pedometers to monitor training step rate.	Participants were instructed to maintain physical activity habits and attended bi-weekly health education sessions that were held separately to those attended by the intervention groups.	Participants were instructed to make no changes to their diet throughout the duration of the study.

*Study did not include 'standard care' or 'no intervention' group. Patients were randomised to either exercise or dietary interventions; ‡ Manuscript refers to same study as van der Heijden *et al* (2009).

10.12 Appendix XII: Details of pooled hepatokine analyses

10.12.1 Methods

FGF21, follistatin and fetuin-A were measured in each laboratory-based study of this thesis (Chapters 4, 5 and 6), whilst LECT2 was investigated in Chapters 4 and 6 only. Samples pre-treated with lithium heparin, which are required for the LECT2 ELISA used in this thesis, were not collected in Chapter 5. SeP was measured in Chapter 4 only and therefore could not be included pooled analyses.

Fasted plasma hepatokine concentrations and other common descriptive variables were pooled from each laboratory-based experimental chapter and were assessed for their suitability for parametric statistical testing as outlined in General Methods (*see Section 3.16*). Due to heterogeneous variances between groups, Kruskal-Wallis tests were used to explore differences in participant characteristics and fasted hepatokine concentrations between the groups of individuals recruited in each chapter. The two groups in Chapter 4 were included separately. Significant main effects were explored *post hoc* using Mann-Whitney U tests and *P*-values were adjusted for multiple comparisons using the Holm-Bonferroni correction (Holm, 1979). Pearson's and Spearman's correlations were performed, as appropriate, to explore relationships between fasted hepatokine concentrations and markers of adiposity or insulin resistance, as well as circulating lipids (TG and total cholesterol) and cardiorespiratory fitness.

10.12.2 Results

Participant characteristics of each group are presented in Table 10.8. By design, the individuals in each of the overweight/obese groups had greater BMI, BF% and WC, compared to the normal weight individuals (Figure 10.1a-b; all *post-hoc* pairwise comparisons $P \leq 0.004$). BF% was greater still in the individuals with overweight or obesity and dysglycaemia compared to those with overweight or obesity alone ($P = 0.045$) and circulating lipids were also higher in each of the overweight/obese groups ($P < 0.04$). HOMA-IR was greater in individuals with dysglycaemia or NALFD compared to those without (Figure 10.1d; $P \leq 0.003$) and there was a progressive increase in Adipo-IR throughout the groups, with the highest value detected in the individuals with NAFLD ($P < 0.04$).

Absolute $\dot{V}O_2$ peak was lower only in the overweight/obese dysglycaemic individuals compared to the other three groups ($P < 0.04$) but these individuals were also significantly older ($P < 0.001$). Conversely, individuals with dysglycaemia or NAFLD had lower relative

$\dot{V}O_2$ peak (normalised to total body weight) compared with the normal weight normoglycaemic participants ($P < 0.01$). It should be noted that relative $\dot{V}O_2$ peak was significantly lower in the overweight/obese normoglycaemic individuals compared to the normal weight normoglycaemic group when these data were analysed in isolation (Chapter 4). Furthermore, in these pooled analyses, the uncorrected *P*-value for the pairwise comparison between these groups was also statistically significant ($P = 0.027$), but became non-significant ($P = 0.08$) after correction for multiple comparisons.

Compared to normal weight individuals, FGF21 was greater in each of the overweight or obese groups (Figure 10.1c; $P \leq 0.024$), but there were no further differences between overweight/obese individuals with normoglycaemia, dysglycaemia or NAFLD ($P \geq 0.81$). As reported in Chapter 4, fasted LECT2 was higher in the overweight/obese participants than the normal weight controls, when all individuals were normoglycaemic ($P = 0.023$). However, fasted concentrations in individuals with NAFLD were not significantly different from either of the normoglycaemic groups (unadjusted $P = 0.07$). Conversely, fasted concentrations of follistatin were greater in individuals with dysglycaemia or NAFLD (Figure 10.1e; $P < 0.0001$) but, as presented in Chapter 4, there were no differences between normal weight or overweight/obese individuals with normal glycaemic control ($P = 0.19$). Fasted concentrations of plasma fetuin-A were greater in individuals with dysglycaemia compared to overweight/obese individuals with normal glycaemic control (Figure 10.1f; $P = 0.01$) but there were no other differences between groups, including the normal weight individuals (unadjusted $P \geq 0.07$).

Table 10.9 presents details of significant correlations between fasted circulating hepatokine concentrations (FGF21, follistatin and fetuin-A only) and markers of cardiometabolic health. Circulating concentrations of FGF21, follistatin and fetuin-A were positively associated with one another and each were positively correlated with markers of adiposity (BMI, WC and/or BF%). There were no relationships between fasted hepatokine concentrations and fasted circulating glucose ($|r|$ or $|rho| \leq 0.26$, $P \geq 0.10$) and only follistatin was positively correlated with circulating NEFA. However, each hepatokine was positively associated with circulating insulin and, in turn, with HOMA-IR and Adipo-IR (all $P < 0.01$). Circulating fetuin-A was negatively associated with absolute $\dot{V}O_2$ peak, whilst the relationships with follistatin and FGF21 approached statistical significance (Table 10.9). Each hepatokine was, however, significantly negatively correlated with $\dot{V}O_2$ peak normalised relative to body weight.

Table 10.8 Participant characteristics, including fasted circulating hepatokines, in participants recruited in exercise laboratory-based experimental chapter of this thesis

	Chapter 4 Normal Weight Normoglycaemic	*Chapter 4* Overweight/Obese Normoglycaemic	*Chapter 5* Overweight/Obese Dysglycaemic	*Chapter 6* Overweight/Obese NAFLD	*P-value*
n	11	11	12	9	
Sex (M/F)	11/0	11/0	6/6	9/0	
Age (years)	30 (24 – 52)	51 (33 – 56)	69 (67 – 71) $^{\gamma}$	39 (35 – 50)	**<0.001**
Anthropometry					
BMI ($kg \cdot m^{-2}$)	23.4 (22.4 – 24.0) $^{\alpha}$	29.2 (27.0 – 31.5)	28.8 (28.4 – 32.5)	32.0 (29.1 – 33.6)	**<0.001**
Body fat (%)	16.9 ± 3.6 $^{\alpha}$	26.4 ± 4.0	35.7 ± 6.0 $^{\gamma}$	28.7 ± 3.0	**<0.001**
Waist circumference (cm)	81.6 ± 5.3 $^{\alpha}$	96.0 ± 7.8	101.7 ± 6.4	111.3 ± 7.5 $^{\delta}$	**<0.001**
Insulin Sensitivity					
Fasted circulating glucose ($mmol \cdot L^{-1}$)*	4.9 ± 0.2	5.0 ± 0.3	5.4 ± 0.5 $^{\varepsilon}$	4.7 ± 0.3	**0.002**
Fasted plasma NEFA ($mmol \cdot L^{-1}$)	0.40 ± 0.18	0.58 ± 0.14	0.51 ± 0.16	0.59 ± 0.14	0.06
Fasted circulating insulin ($mU \cdot L^{-1}$)*	26.1 (22 – 33.8)	32.9 (23.0 – 39.1)	63.7 (50.5 – 99.1) $^{\gamma}$	101.2 (83.3 – 127.7) $^{\delta}$	**<0.001**
HOMA-IR	0.95 ± 0.39	1.21 ± 0.67	2.89 ± 1.19 $^{\zeta}$	3.67 ± 0.96 $^{\zeta}$	**<0.001**
Adipo-IR	12.6 ± 9.0 $^{\alpha}$	22.0 ± 13.0 $^{\beta}$	35.2 ± 12.5 $^{\gamma}$	61.8 ± 20.4 $^{\delta}$	**<0.001**
Cardiorespiratory Fitness					
Absolute $\dot{V}O_2$ peak ($L \cdot min^{-1}$)	3.46 ± 0.74	3.21 ± 1.21	2.19 ± 0.50 $^{\gamma}$	3.23 ± 0.41	**0.001**
Relative $\dot{V}O_2$ peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	50.1 ± 11.9	38.5 ± 9.7	26.5 ± 4.6 $^{\eta}$	31.8 ± 4.8 $^{\eta}$	**<0.001**
Circulating Lipids					
Triglyceride ($mmol \cdot L^{-1}$)	0.89 (0.64 – 1.33) $^{\alpha}$	1.79 (1.11 – 2.31)	1.36 (1.19 – 2.31)	2.18 (1.27 – 2.60)	**0.004**
Total Cholesterol ($mmol \cdot L^{-1}$)	4.12 ± 0.73 $^{\alpha}$	4.91 ± 0.89	5.18 ± 0.91	4.88 ± 0.59	**0.034**
Hepatokines					
FGF21 ($pg \cdot mL^{-1}$)	64 (32 – 144) $^{\alpha}$	183 (133 – 266)	143 (133 – 386)	176 (110 – 505)	**0.007**
Follistatin ($pg \cdot mL^{-1}$)	795 ± 257	670 ± 154	2077 ± 480 $^{\zeta}$	2381 ± 909 $^{\zeta}$	**<0.001**
Fetuin-A ($\mu g \cdot mL^{-1}$)	541 ± 137	497 ± 99	682 ± 141 $^{\theta}$	608 ± 138	**0.016**
LECT2 ($ng \cdot mL^{-1}$)	30.8 ± 9.9 $^{\alpha}$	47.6 ± 17.2	Not Measured	40.0 ± 4.9	**0.023**

*In Chapter 4 and 5, glucose and insulin are measured in plasma. In Chapter 6, glucose and insulin were measured in whole blood and serum, respectively. $^{\alpha}$ normal weight normoglycaemic group significantly different from each other group ($P < 0.05$); $^{\beta}$ overweight/obese normoglycaemic group significantly different from each other group ($P < 0.05$); $^{\gamma}$ overweight/obese dysglycaemic group significantly different from each other group ($P < 0.05$); $^{\delta}$ overweight/obese NAFLD group significantly different from each other group ($P < 0.05$); $^{\varepsilon}$ significantly different from normal weight normoglycaemic and overweight/obese NAFLD groups ($P < 0.05$); $^{\zeta}$ significantly different from both normoglycaemic groups ($P < 0.05$); $^{\eta}$ significantly different from normal weight normoglycaemic group ($P < 0.01$); $^{\theta}$ significantly different from overweight/obese normoglycaemic group.

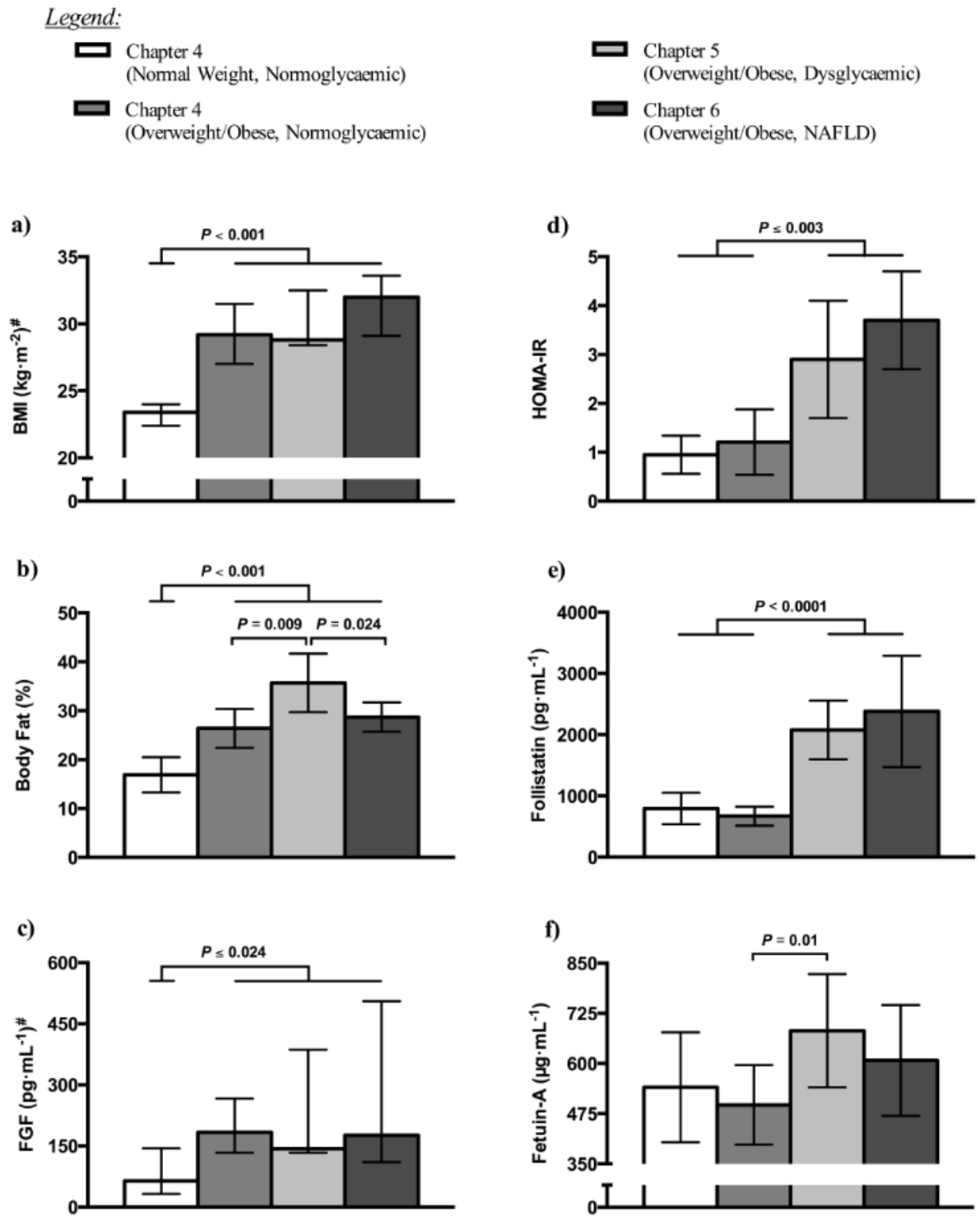


Figure 10.1 Comparisons of adiposity, insulin resistance and fasted hepatokine concentrations in laboratory-based experimental studies (Chapters 4, 5 and 6). Data presented as mean ± SD unless otherwise stated; # indicates that one of more groups were not normally distributed and thus data presented as median (IQR). To allow consistent presentation, some data may appear as a different summary statistic to that reported in the respective chapter of this thesis.

Table 10.9 Statistically significant relationships (and selected others) between fasted hepatokines concentrations and makers of cardiometabolic health in the pooled population of individuals recruited in this thesis.

	Fasted Circulating FGF21 *Correlation Co-efficient (P-value)*	**Fasted Circulating Follistatin** *Correlation Co-efficient (P-value)*	**Fasted Circulating Fetuin-A** *Correlation Co-efficient (P-value)*
Age		*rho* = 0.37 (*P* = 0.014)	
Anthropometry	~	~	~
Body weight	*r* = 0.57 (*P* < 0.001)		
BMI	*r* = 0.58 (*P* < 0.001)	*rho* = 0.21 (*P* = 0.009)	
BF%	*r* = 0.47 (*P* = 0.002)	*rho* = 0.52 (*P* < 0.001)	*r* = 0.43 (*P* = 0.005)
WC	*r* = 0.66 (*P* < 0.001)	*rho* = 0.54 (*P* < 0.001)	
Insulin Sensitivity	~	~	~
Fasted Circulating Glucose			
Fasted Circulating Insulin	*rho* = 0.46 (*P* = 0.002)	*rho* = 0.70 (*P* < 0.001)	*r* = 0.46 (*P* = 0.002)
HOMA-IR	*rho* = 0.46 (*P* = 0.002)	*rho* = 0.71 (*P* < 0.001)	*r* = 0.45 (*P* = 0.003)
NEFA	*r* = 0.55 (*P* < 0.001)		
Adipo-IR	*r* = 0.67 (*P* < 0.001) [#]	*rho* = 0.65 (*P* < 0.001)	*r* = 0.42 (*P* = 0.006) [#]
Circulating Lipids	~	~	~
Fasted Plasma Triglyceride	*r* = 0.47 (*P* = 0.001) [#]	*rho* = 0.35 (*P* = 0.02)	
Total Cholesterol			
Cardiorespiratory Fitness	~	~	~
Absolute $\dot{V}O_2$ peak	[*rho* = -0.30 (*P* = 0.053)]	[*rho* = -0.28 (*P* = 0.071)]	*r* = -0.34 (*P* = 0.003)
Relative $\dot{V}O_2$ peak	*r* = -0.59 (*P* < 0.001)	*rho* = -0.44 (*P* = 0.003)	*r* = -0.36 (*P* = 0.002)
Hepatokines	~	~	~
Fasted Circulating FGF21	~	*rho* = 0.36 (*P* = 0.02)	*r* = 0.36 (*P* = 0.02) [#]
Fasted Circulating Follistatin	~	~	*rho* = 0.51 (*P* = 0.001)

Shaded areas represent no significant correlations between variables. FGF21 and fetuin-A data were log transformed prior to parametric analyses; follistatin data were not normally distributed even when log transformed so non-parametric analyses were performed; [#] indicates independent variable was log transformed prior to analysis.