UNIVERSITY OF HULL

Exercise, Postprandial Lipids and Risk of Non-Alcoholic Fatty Liver Disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver disorders from fatty accumulation to non-alcoholic steatohepatitis (NASH). The worldwide prevalence of NAFLD is estimated to be 30% and it is frequently observed in those living with obesity and Type 2 Diabetes Mellitus (T2DM). Despite this there is an apparent gap between the numbers diagnosed and the prevalence within the general population. Composite scoring panels consisting of routinely acquired anthropometric and biochemical variables provide a convenient method to identify those at risk of NAFLD and disease progression. In addition to health care settings, these panels could also be used for research purposes in population-based observational studies. The first chapter of this thesis therefore sought to estimate the prevalence of NAFLD and hepatic fibrosis in a retrospective cross-sectional cohort study of apparently healthy young to middle-aged adults using several NAFLD composite scoring panels. A second aim of the study was to examine the cross-sectional associations between selected lifestyle variables and other predictors of metabolic risk with estimates of hepatic steatosis and fibrosis using structural equation modelling (SEM).

Using the Fatty Liver Index (FLI) (Bedogni et al., 2006) the estimated prevalence of NAFLD was 34.9% within a cohort of 364 apparently healthy adults. The prevalence and severity of hepatic fibrosis varied significantly according to the composite fibrosis scoring panel selected and cut-off applied. In those participants with presumed NAFLD (FLI $\geq$ 60), the estimated prevalence of fibrosis was typically 20-50%; although estimates of advanced fibrosis were lower and more variable between panels. The SEM analysis identified the latent variable of metabolic syndrome (MetS) diagnosis as a predictor of liver fat ($p < 0.001$); and total and regional adiposity measures, as indicated by body mass index (BMI) and waist circumference (WC) as predictors of hepatic fibrosis ($p < 0.001$).

Both BMI and WC (in conjunction with routine lipid and liver function tests [LFTs]) may be used as simple non-invasive measures to identify those individuals at a higher risk of hepatic fibrosis. These anthropometric measures were therefore used to inform the inclusion criteria for Chapter 5 and Chapter 6, respectively.

Lifestyle changes, including dietary modifications and increased physical activity/structured exercise, are advocated for the management of NAFLD. In terms of specific dietary factors, fructose consumption has been associated with poor metabolic health and acutely increases postprandial triglyceride (TG) concentrations, a risk factor for cardiometabolic disease, including NAFLD. Conversely, acute exercise is an established method to attenuate disturbances in postprandial TG and glucose metabolism. Clinical and research evaluation of postprandial lipid metabolism has largely focused on the utility of the oral fat tolerance test (OFTT) to best exemplify risk of NAFLD and atherosclerotic vascular disease. The purpose of Chapter 5 was therefore to investigate the acute ingestion of an alternative oral metabolic challenge; namely fructose included within an OFTT. This study also examined the influence of prior evening exercise on postprandial metabolism in apparently healthy men. As informed by Chapter 4, non-obese, physically active males were recruited (sedentary participants and those with clinical obesity [BMI $\geq$ 30 kg·m^{-2} or WC $\geq$ 94 cm] were excluded from the study). Following a screening visit, eight adult males (age [median, IQR]: 25 [2] years, BMI [mean, SD]: 25.1 [1.7] kg·m^{-2}, WC [mean, SD]: 85.4 [4.2] cm, and self-reported physical activity > 150 minutes per week) ingested an OFTT with the addition of fructose (OFTT-Fruc; 73 g fat, 60 g fructose, 14 g protein) or sucrose (OFTT-Sucr; 73 g fat, 60 g sucrose, 14 g protein) on four separate morning visits. Volunteers rested or performed supervised prior evening exercise (40 minutes submaximal high intensity interval exercise, [HIIE]) the evening before each OFTT. OFTT-Fruc significantly increased the TG integrated area under the

curve (iAUC) ($p = 0.024$, partial eta squared [ηp^2] = 0.542) compared to OFTT-Sucr. Prior evening HIIE did not attenuate the TG response to either OFTT-Fruc or OFTT-Sucr.

The final experimental chapter of this thesis extended the methods utilised in Chapter 5 and included individuals at higher risk of MetS and NAFLD. Following a screening visit, five inactive, overweight and centrally obese adult males (age [median, IQR]: 54 [20] years, BMI [mean, SD]: 32.7 [4.2] $kg \cdot m^{-2}$, WC [mean, SD]: 113.9 [13.3] cm) and self-reported physical activity < 150 minutes per week) ingested either OFTT-Fruc or OFTT-Sucr on four separate days as outlined above. Similarly, participants either rested or performed supervised prior evening HIIE the evening before each OFTT. OFTT-Fruc significantly increased the TG total area under the curve (tAUC) ($p = 0.010$, $\eta p^2 = 0.838$) compared to OFTT-Sucr. There was a trend towards statistical significance and a large effect size for the lowering of the TG tAUC (by approximately 12%) following prior evening HIIE ($p = 0.067$, $\eta p^2 = 0.610$).

The primary outcomes of this thesis demonstrate that the likely prevalence of NAFLD in an apparently healthy cross-sectional cohort of self-selected adults attending for preventive health assessment is in accordance with previous estimates from population-based surveys. Namely, participants with presumed NAFLD (as defined by an FLI ≥ 60), were predominantly male (90.3%), older to middle-aged, clinically and abdominally obese (with significantly higher BMI, body fat content and enlarged WC). They were evidently dyslipidaemia with higher blood pressure and a higher prevalence of MetS diagnosis (up to 60%) compared to those individuals with an FLI < 10 ($p < 0.001$ for all above cited variables, respectively). Consistently, they were more likely to be inactive with lower cardiorespiratory fitness (predicted maximal oxygen uptake [$\dot{V}O_{2max}$]). Likewise, liver enzymes, especially gamma-glutamyl transferase (GGT), were significantly higher ($p < 0.001$) but typically within the routine normal reference range and participants self-reported comparable modest alcohol consumption (median [IQR]:

10 [11] units per week). The predicted prevalence of hepatic fibrosis was inconsistent from biopanel estimates ranging from 0% to 77.17% depending on the complexity of the composite panel and cut-off applied. Furthermore, BMI and WC may be utilised with routine lipids and LFTs to identify those with NAFLD at a higher risk of hepatic fibrosis. These simple measures could be easily integrated into the primary care setting to identify those requiring referral for specialist hepatic medical imaging. The risk of NAFLD and NASH/hepatic fibrosis has been strongly associated with disturbances in postprandial lipid metabolism and sub-clinical inflammatory/oxidative processes. This thesis also examined the influence of fructose included within an OFTT. The inclusion of fructose within an OFTT produced a significantly higher TG response in both apparently healthy lean physically active males and overweight/centrally obese inactive adult males. In those at risk of MetS and NAFLD, prior evening HIIE attenuated post-challenge TG responses the following morning; an effect not observed in those with lower NAFLD risk status (normolipidaemia, non-obese and more active adult males).

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List of Abbreviations

Acetyl Co-A; Acetyl coenzyme-A

ALD; Alcoholic liver disease

ALT; Alanine aminotransferase

ANOVA; Analysis of variance

Apo; Apolipoprotein

APRI; Alanine platelet ratio index

ARFI; Acoustic radiation force impulse imaging

ASH; Alcoholic steatohepatitis

AST; Aspartame aminotransferase

AST:ALT; Alanine aminotransferase ratio

AT; Anaerobic threshold

ATGL; Adipose tissue triglyceride lipase

ATP; Adenosine tri-phosphate

AUC; Area under the curve

AUROC; Area under the receiver operator curve

BARD; body mass index, alanine aminotransferase ratio, and diabetes score

BMI; Body mass index

cADPR; cyclic ADP-ribose

Calcium; Ca^{2+}

CFI; Comparative fit index

ChREBP; Carbohydrate-responsive element-binding protein

cm; Centimetre

CO_2; Carbon dioxide

CPET; Cardiopulmonary exercise test

CVD; Cardiovascular disease

DHAP; Di-hydroxy-acetone-phosphate

DNL; *De novo* lipogenesis

EDTA; Ethylenediaminetetraacetic acid

FIB-4; Fibrosis 4

FLD; Fatty liver disease

FLI; Fatty liver index

FPG; Fasting plasma glucose

g; Grams

GGT; gamma-glutamyl transferase

GAP; Glyceraldehyde-3-phosphate

GLUT; Glucose transporter

HDL; High density lipoproteins

HDL-c; High density lipoprotein cholesterol

HFCS; High fructose corn syrup

HIIE; High intensity interval exercise

HOMA-IR; Homeostatic model of insulin resistance

HR; Heart rate

HSL; Hormone sensitive lipase

iAUC; Incremental area under the curve

IDL; Intermediate density lipoprotein

IHTG; Intrahepatic triglyceride content

IP3; $_{D}$-*myo*-inositol 1,4,5-trisphosphate

IQR; Inter-quartile range

Kcal; Kilocalorie

Kg; Kilogram

KHK-C; Ketohexokinase C

l; Litre

LAP; Lipid accumulation product

LDL; Low density lipoproteins

LDL-c; Low density lipoprotein cholesterol

LFTs; Liver function tests

LPL; lipoprotein lipase

MAFLD; Metabolic-dysfunction associated fatty liver disease

MASLD; Metabolic-dysfunction associated steatotic liver disease

mbar; Millibar

MCAR; Missing completely at random

MD; Mean difference

MIE; Moderate intensity exercise

MJ; Megajoule

μl; Microlitre

mmol; Millimole

MetS; Metabolic syndrome

mg; Microgram

min; Minute

ml; Millilitre

mmHg; Millimeters of Mercury

MRE; Magnetic resonance elastography

MRI; Magnetic resonance imaging

MRI PDFF; Magnetic resonance imaging proton density fat fraction

MRS; Magnetic resonance spectroscopy

^{1}H-MRS; Proton magnetic resonance spectroscopy

n; number of participants

O_2; Oxygen

NEFAs; Non esterified fatty acids

NFS; Non-alcoholic fatty liver disease fibrosis score

NHS; National Health Service

NAFL; Non-alcoholic fatty liver

NAFLD; Non-alcoholic fatty liver disease

NPV; Negative predictive value

OFTT; Oral fat tolerance test

OFTT-Fruc; Oral fat tolerance test with fructose

OFTT-Sucr; Oral fat tolerance test with sucrose

OFTT-Fruc-Ex; Oral fat tolerance test with fructose following prior exercise

OFTT-Sucr-Ex; Oral fat tolerance test following sucrose with prior exercise

OGTT; Oral glucose tolerance test

p; Probability value

PHE; Public Health England

PPAR-γ; Peroxisome proliferator-activated receptor- γ

PPV; Positive predictive value

RER; Respiratory exchange ratio

RMR; Resting metabolic rate

RMSEA; Root-mean-square of approximation

ROC; Receiver operator curve

RPE; Rating of perceived exertion

SAF; Steatosis, activity and fibrosis score

SD; Standard deviation

SEM; Structural equation modelling

SGLT1; Sodium dependant glucose transporter 1

SREBP-1c; Sterol regulatory element binding protein 1c

SRMSR; Standardised root-mean-square residual

tAUC; Total area under the curve

TC; Total cholesterol

T2DM; Type 2 Diabetes Mellitus

TG; Triglyceride

TLI; Tucker-Lewis index

TRL; Triglyceride rich lipoproteins

TSRA; Time series response analyser

US; Ultrasound

VCAM-1; Vascular cell adhesion molecule-1

VLDL; Very low density lipoprotein

$\dot{V}e$: Rate of ventilation

$\dot{V}O_2$; Rate of oxygen uptake

$\dot{V}O_{2max}$; Maximal oxygen uptake

$\dot{V}O_{2peak}$; Peak oxygen uptake

W; Watt

χ^2; Chi squared

95% CI; 95% confidence interval

°C; Degrees Celsius

ηp^2; Partial eta squared

WC; Waist circumference

WHR; Waist to hip ratio

WHtR; Waist to height ratio

Chapter 1 General Introduction

1.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver disease from fatty infiltration to NASH (Chalasani et al., 2018; Marchesini et al., 2016). Once thought to be benign, the global prevalence of NAFLD is estimated to be 30% and is the leading cause of liver-related morbidity and mortality (Younossi et al., 2023). The emergence of NAFLD has paralleled that of obesity and T2DM (Targher et al., 2021; Younossi, 2019) and is considered the hepatic manifestation of MetS. This has been reflected more recently with a proposed change in terminology from NAFLD to 'metabolic-dysfunction associated fatty liver disease' (MAFLD) (Eslam et al., 2020a), to further emphasise the strong association and frequent co-existence of these diseases. Despite this, there is a gap in the prevalence of NAFLD and the numbers diagnosed, particularly in primary care, where NAFLD is under-recognised and often an incidental finding (Alexander et al., 2018; Armstrong et al., 2012). This has necessitated the development of several minimally invasive composite scoring panels to identify those at risk whilst avoiding unnecessary referrals to secondary care (Castera et al., 2019). However, little is known about the utility of these panels in determining the prevalence of NAFLD and hepatic fibrosis away from healthcare or clinical research settings; with limited data from observational studies (Kim et al., 2013; Long et al., 2016; Sesti et al., 2014). This has therefore precluded the large-scale population-based study of NAFLD.

Regarding management, lifestyle changes including diet and exercise are advocated for those living with NAFLD, similar to other co-morbidities (Hallsworth & Adams, 2019). It has been suggested that fructose consumption may be associated with poor metabolic health, including the development of NAFLD (Campos & Tappy, 2016; Tappy, 2018). Despite the ergogenic effects of fructose to improve endurance exercise performance

when included with glucose (Jeukendrup, 2010), fructose consumption also increases postprandial TG concentrations at rest (Chong et al., 2007); a risk factor for cardiometabolic disease (Kolovou et al., 2019; Mihas et al., 2011). Contrary to the traditional use of fasting measurements to determine metabolic dysfunction, guidance now also advocates the use of postprandial measurements (Nordestgaard et al., 2016). Following the consumption of a mixed nutrient meal, blood glucose and TG concentrations are raised for approximately two and eight hours, respectively (Edinburgh et al., 2017). Based on the consumption of at least three meals per day, the majority of people spend their waking hours in the postprandial state (Edinburgh et al., 2017). Postprandial glucose and lipid measurements therefore better reflect habitual eating patterns.

Typically, postprandial metabolic excursions have been measured in response to a nutritional challenge, such as the oral glucose tolerance test (OGTT) (Alberti et al., 1985) or OFTT (Kolovou et al., 2011). However, individuals rarely consume macronutrients in isolation. Mixed nutrient metabolic challenges have therefore been proposed (Mohanlal & Holman, 2004; Stroeve et al., 2015). The addition of fructose or fructose containing carbohydrates to an oral fat load exacerbates postprandial TG concentrations (Cohen & Schall, 1988; Grant et al., 1994). This is important as pronounced postprandial glucose and TG excursions are associated with cardiovascular and metabolic diseases (Edinburgh et al., 2017). Acute exercise is one strategy to attenuate postprandial metabolic dysfunction (Burns et al., 2015; Freese et al., 2013). However, these nutrition and exercise interactions require further investigation, particularly in those at risk of cardiometabolic disease, including NAFLD.

Aims and objectives of the thesis:

1. To determine the prevalence of NAFLD and hepatic fibrosis in a retrospective cohort study of self-selected apparently healthy young to middle-aged adults using several widely recommended and utilised NAFLD composite scoring panels (Chapter 4).
2. A second aim of the study was to examine the cross-sectional association between lifestyle variables and predictors of metabolic risk, with estimates of hepatic steatosis and fibrosis (Chapter 4).
3. To evaluate different methodologies with respect to the determination of the postprandial lipid responses to a lipid challenge/OFTT with the addition of different forms of carbohydrate. Specifically, to compare the postprandial metabolic effects of fructose and sucrose when included in an OFTT and the efficacy of acute prior exercise to attenuate metabolic responses in apparently healthy non-obese adults (Chapter 5).
4. To compare the postprandial metabolic effects of fructose and sucrose when included in an OFTT and the efficacy of acute prior exercise to attenuate metabolic responses in inactive, overweight and obese adults at higher risk of NAFLD (Chapter 6).

Hypothesises related the above aims and objectives:

1. The prevalence of NAFLD as determined by the FLI would be largely consistent with existing regional and global estimates. However, the estimated prevalence and severity of fibrosis and advanced fibrosis would be highly variable depending on the complexity of the predictive panel utilised.
2. Established risk factors, including overweight and obesity, in addition to components of MetS, would be predictive of NAFLD and hepatic fibrosis (as determined by surrogate panels) in a retrospective analysis of apparently healthy young to middle-aged adults.
3. The addition of fructose to an OFTT would significantly increase the postprandial TG response (compared to sucrose) in apparently healthy, physically active individuals. Furthermore, a prior evening exercise session would attenuate this response.
4. The addition of fructose to an OFTT would similarly increase the post challenge TG response (compared to sucrose) in sedentary, overweight and obese adults with components of MetS. It was hypothesised that prior evening exercise would again attenuate this response.

Chapter 2 will introduce pertinent literature and concepts related to: NAFLD and diagnostic methods, including composite scoring panels for hepatic steatosis and fibrosis; fructose metabolism and cardiometabolic disease risk; and exercise a strategy to attenuate postprandial dysmetabolism.

Chapter 2 Review of Literature

2.1 Purpose and overview

This chapter provides a detailed yet concise narrative review of the literature pertinent to the experimental chapters presented in this thesis. The body of work cited is intended to be thorough, however, it is not exhaustive. Recommendations for further reading are provided where a comprehensive review of a topic is available (i.e. systematic review and/or meta-analysis) or is beyond the scope of the current chapter.

2.2 Basic structure and function of the liver

The word liver originates from old Norse ‘lifr’ and the term ‘hepatic’ pertaining to the liver from the Greek ‘hepatos’ (Frayn & Evans, 2019). The mass of the adult human liver is approximately 1-1.5 kg, representing 2% of total body mass and is located in the upper right quadrant; immediately inferior to the diaphragm (Frayn & Evans, 2019; Schaffner & Popper, 1985). The anatomic position and proximity of the liver to other splanchnic organs reflect its central role in metabolic homeostasis. Blood is supplied by a dual system comprising of the hepatic artery and hepatic portal vein. Uniquely, the liver receives the majority of its blood flow from the latter (Frayn & Evans, 2019; Mccuskey, 2008). Although oxygen poor having circulated the splanchnic capillary bed, the blood supplied by the (hepatic) portal vein is rich in nutrients absorbed from the intestinal lumen and hormones secreted by the pancreas (Arias et al., 2020; Frayn & Evans, 2019). These hydrophilic nutrients include simple sugars (monosaccharides) and proteins (amino acids), in addition to the hormones insulin and glucagon from the exocrine cells of the pancreas (Frayn & Evans, 2019). These hormones are fundamental to intra and extrahepatic carbohydrate and protein metabolism. Blood exits the liver via several hepatic veins to

the inferior vena cava for venous return to the heart (Frayn & Evans, 2019; McCuskey, 2008).

A further efferent network of vessels transports bile from the liver to the gallbladder (Frayn & Evans, 2019). Bile is comprised predominantly of water (~ 95%) plus organic solutes, inorganic electrolytes and proteins (Arias et al., 2020). The detergent action of the bile salts (contained within bile) is essential for the digestion and absorption of hydrophobic nutrients, including fat (lipids) and fat-soluble vitamins (Frayn & Evans, 2019; Jenkins & Billing, 1985).

The structure of the liver consists primarily of five specialised cell types (Juza & Pauli, 2014). The most abundant and well studied are the parenchymal hepatocytes that appear in a hexagonal formation when viewed as a cross-section (Arias et al., 2020; Frayn & Evans, 2019, Figure 2.1). This formation constitutes the hepatic lobules which have a micro-vascular structure comprised of the central hepatic vein and a triad of vessels at each adjoining corner, including branches of the aforementioned hepatic artery and portal vein, as well as the bile duct (Arias et al., 2020; Frayn & Evans, 2019; Juza & Pauli, 2014). Hepatocytes are stacked in a plate like fashion radiating out from the central vein with the triads forming vascular columns (Arias et al., 2020; Frayn & Evans, 2019). Arterial and portal venous blood flows centripetally from the periphery through tributaries known as sinusoids (intrahepatic equivalent of capillaries), bathing the hepatocytes, before draining into the central vein and joining the systemic circulation in the inferior vena cava (Arias et al., 2020; Frayn & Evans, 2019; McCuskey, 2008). In contrast, bile secreted by the hepatocytes flows in the opposing centrifugal direction and instead exits via the bile duct for storage in the gallbladder (Arias et al., 2020; Frayn & Evans, 2019).

This is just one example of the myriad of complex metabolic tasks hepatocytes perform, in addition to glucose, fatty acid, and amino acid metabolism. However, hepatocytes are not a homogenous cell population with their function dictated according to their location in the parenchyma (Arias et al., 2020; Frayn & Evans, 2019; Schaffner & Popper, 1985). The latter is sectorised into periportal, intermediate and pericentral zones according to the proximity to the aforementioned vascular structures (i.e. portal vein and hepatic artery or central vein, respectively) (Frayn & Evans, 2019; Jungermann & Keitzmann, 1996; Schaffner & Popper, 1985). Periportal hepatocytes are perfused with blood arriving at the liver from the portal vein and hepatic artery and are therefore reasonably well supplied with oxygen and substrates. Their function is reflective of this extracellular milieu in which oxidative metabolism predominates energy transfer. Glucose synthesis (gluconeogenesis) therefore occurs mainly in these cells. In contrast, hepatocytes in the pericentral zone are involved mainly in glycolysis and ketone body synthesis due to the declining oxygen saturation and increasing anaerobic environment (Frayn & Evans, 2019; Kietzmann, 2017; Schaffner & Popper, 1985). This highly organised structure of extraction is termed ‘metabolic zonation’ (Frayn & Evans, 2019; Jungermann & Keitzmann, 1996).

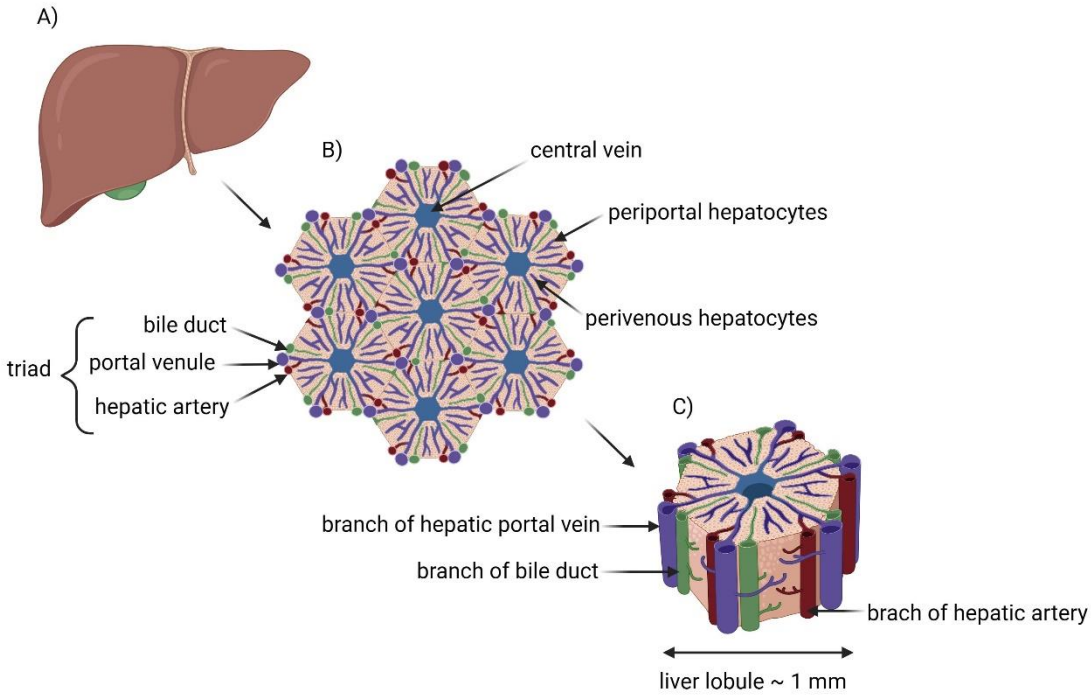

Figure 2.1 Arrangement of hepatocytes in liver lobules. A) The liver including gallbladder. B) Arrangement of liver lobules. C) Individual lobule. Adapted from Frayn & Evans (2019). Created with BioRender.com

2.3 The liver: the chief metabolic organ

As described in section 2.2 the liver is responsible for a variety of metabolic functions. Pertinent to the thread of the current thesis are those pathways involved in carbohydrate and lipid metabolism. For example, in the fed or postprandial state, the liver is one of the first tissues exposed to ingested carbohydrate and is the primary site of fructose metabolism in humans (Gonzalez & Betts, 2018; Gonzalez & Betts, 2019; Pinnick & Hodson, 2019). Similarly, the liver receives, synthesises, stores and mobilises several lipid fuels in the transition from the fasted to fed state (Hodson & Fielding, 2010; Hodson & Gunn, 2019a). The presentation of these macronutrients to the hepatocytes therefore poses an acute metabolic challenge.

The ability to maintain blood glucose and lipid homeostasis in the postprandial state is characteristic of good metabolic health (Edinburgh et al., 2017; van Ommen et al., 2014).

Following the consumption of a mixed-nutrient meal, blood glucose and TG concentrations remain elevated for approximately two and eight hours, respectively (Fery et al., 1990; Frayn, 1997; Frayn & Evans, 2019). However, the time-course and extent (peak) of these metabolic responses may be exacerbated in the presence of insulin resistance (McQuaid et al., 2011; Reaven, 1988); which appears to be the primary link between obesity and several non-communicable disease states, including NAFLD (Armstrong et al., 2014; Byrne & Targher, 2015; Kitade et al., 2017).

2.3.1 Hepatic glucose metabolism in the transition from the fasted to fed state

In apparently healthy humans, blood glucose concentration in the fasted or postabsorptive resting state is tightly governed at 4-5 $mmol \cdot l^{-1}$ (Edinburgh et al., 2017; Wasserman, 2009), despite a high rate of turnover of approximately 2 $mg \cdot kg \cdot min^{-1}$ as glucose enters and exits the blood for use by extrahepatic tissues, including the brain, skeletal muscle and heart (Edinburgh et al., 2017; Fery et al., 1990; Frayn & Evans, 2019). Glycogen stored within hepatocytes is liberated by glycogenolysis via the enzymatic action of glycogen phosphorylase and regulated by glucagon (Frayn & Evans, 2019). The liver also receives glucogenic substrates, including pyruvate, lactate and alanine from peripheral tissues to synthesise glucose endogenously (gluconeogenesis) (Frayn & Evans, 2019). Therefore, the ability of the liver to coordinate glucose release and synthesis from both of these pathways has consequences for metabolic control and health (Gonzalez & Betts, 2019).

Following the consumption of a carbohydrate-rich meal of approximately 60-80 g, assuming a mean carbohydrate intake of 200-240 g per day (Scientific Advisory Committee on Nutrition [SACN], 2015) across three to four eating occasions (Wittig et al., 2017), blood glucose concentration will peak at 7-8 $mmol \cdot l^{-1}$ in healthy individuals. This represents a rise of 60% from the postabsorptive state (Frayn et al., 1993; Frayn &

Evans, 2019) before returning to homeostatic range within one to two hours postprandially (Fery et al., 1990; Frayn, 1997). This metabolic response is attenuated by the suppression of hepatic glucose output by glucagon and in turn glycogen phosphorylase is down regulated and the activity of glycogen synthase increases in response to insulin (Fery et al., 1990; Taylor et al., 1996). Concomitantly, glucose uptake in extrahepatic tissues, primarily skeletal muscle, is increased (DeFronzo et al., 1985; Taylor et al., 1993).

The remaining monosaccharides, galactose and fructose, are distinctively metabolised within the liver (Gonzalez & Betts, 2019; Tappy & Lê, 2010). In comparison to galactose, the metabolism of fructose is well described in the pentose pathway (Gonzalez & Betts, 2019; Tappy & Lê, 2010). The majority of fructose is converted via gluconeogenesis to glucose (~ 50%) and glycogen (15-25%); however one further fate is the conversion to fatty acids via *de novo* lipogenesis (DNL) (Gonzalez & Betts, 2019; Tappy & Lê, 2010). DNL occurs primarily in the liver and is a pathway for disposing of excess non-lipid precursors (monosaccharides and amino acids) (Pinnick & Hodson, 2019; Sun & Empie, 2012). Hepatic fructose metabolism therefore has potential implications for metabolic health (Hengist et al., 2019; Tappy & Lê, 2010), including the development of NAFLD (Jin & Vos, 2015; Moore, 2019; Tappy, 2018). An overview of Fructose metabolism and Non-alcoholic fatty liver disease are discussed in further detail in the corresponding sections below.

2.3.2 Hepatic lipid metabolism in the transition from the fasted to fed state

The liver also plays a central regulatory role in the distribution and storage of lipids in the fasted and fed states (Hodson & Fielding, 2010; Hodson & Gunn, 2019a). As with blood glucose, the concentrations of lipids in plasma are regulated by hepatic and extrahepatic tissues (Frayn et al., 2006; Hodson & Fielding, 2010). However, whilst one primary form

of carbohydrate (glucose) circulates the blood, there are several lipid fuels that vary in concentration throughout the day (Frayn & Evans, 2019; Ruge et al., 2009). Sources include dietary fats, the mobilisation of stored lipids, as well as non-lipid lipogenic substrates such as fructose and lactate that can be converted to fatty acids via DNL (Hodson & Fielding, 2010).

Unlike hydrophilic monosaccharides that are absorbed across the intestinal lumen by pumps and proteins without prior modification, dietary lipid absorption consists of several steps as reviewed by others (Ko et al., 2020; Xiao et al., 2019). Dietary fats are hydrolysed to monoglycerides and fatty acids by pancreatic lipase in the small intestine and form micelles with bile salts, which facilitates their entry at the brush border membrane into the enterocyte (Ko et al., 2020; Xiao et al., 2019). Monoglycerides and fatty acids are re-esterified to TGs within the endoplasmic reticulum of the enterocyte to form cytosolic lipid droplets, or are secreted and packaged for pre-chylomicron synthesis (Ko et al., 2020; Xiao et al., 2019). Following maturation, mature chylomicrons exit the enterocyte into the lamina propria and move through the lymphatic vessels before entering the venous circulation (Ko et al., 2020; Xiao et al., 2019).

Lipids are transported in the form of cholesterol and TGs (Ginsberg et al., 2005). The latter is the primary form of dietary fat consumed and consists of three individual fatty acids each linked by an ester bond to a glycerol head (Frayn & Evans, 2019; Frayn, 2016). Cholesterol and TGs are transported within the core of lipoproteins (proteins containing lipids) which are classified according to their physical-chemical characteristics (Ginsberg et al., 2005). The main classes of lipoproteins are chylomicrons, very low density lipoproteins (VLDLs), low density lipoproteins (LDLs), and high density lipoproteins (HDLs); ranging from largest to smallest based on their size, density, and protein to lipid ratio (Frayn & Evans, 2019; Ginsberg et al., 2005, Figure 2.2). Chylomicrons and VLDLs are rich in TGs and are often referred to as triglyceride rich lipoproteins (TRLs). They are

mainly responsible for transporting TGs to tissues (Frayn & Evans, 2019; Ginsberg et al., 2005). The outer surface of lipoproteins are comprised of free cholesterol, phospholipids, and apolipoproteins. The apolipoprotein family are integral to lipoprotein metabolism. For example, apolipoprotein (Apo) B100 is a prerequisite for the synthesis of hepatically-derived VLDLs. Similarly, Apo B48, a truncated form of Apo B100, is required for chylomicron assembly in the intestine as described above (Ginsberg et al., 2005; Xiao et al., 2019). Non-esterified fatty acids (NEFAs) represent the second major circulating lipid fuel in addition to TG. However, rather than being packaged and carried in lipoproteins, NEFAs are transported in plasma bound to albumin (Frayn & Evans, 2019).

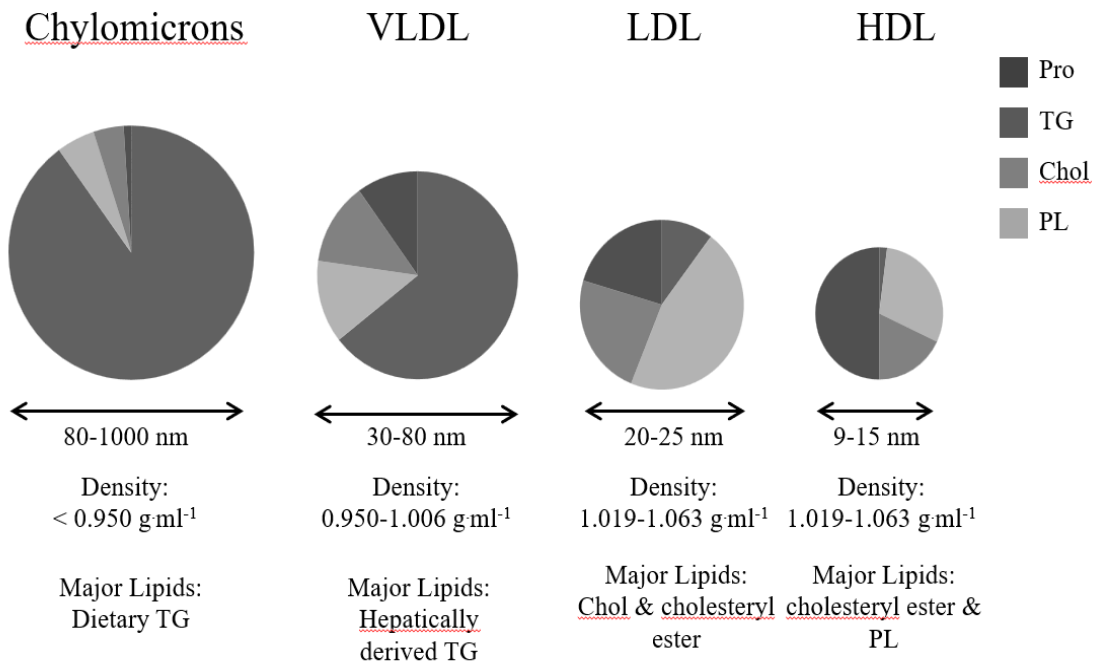

Figure 2.2 Characteristics of the major lipoprotein classes. Composition is percentage by weight. VLDL, very low-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; TG, triglyceride; Chol, cholesterol; PL, phospholipid; Pro, protein; nm, nanometre; $g \cdot ml^{-1}$, grams per millilitre. Adapted from data presented by Frayn & Evans (2019).

In the postabsorptive state, NEFAs are the predominant lipid fuel source entering the liver and are favoured for oxidation (Frayn et al., 2006; Hodson & Frayn, 2011). NEFAs are mixed with the existing intrahepatic pool of TGs and fatty acids synthesised *de novo* from non-lipid precursors (DNL). NEFAs may also be esterified for TG synthesis and stored as lipid droplets within hepatocytes or alternatively assembled within VLDL-TGs for export and delivery to peripheral tissues depending on the prevailing metabolic conditions (Hodson & Fielding, 2010). NEFAs are stored in subcutaneous and visceral adipose tissue in the form of TGs and are de-esterified and liberated into plasma by two major lipolytic enzymes: adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) (Frayn & Evans, 2019; Zechner et al., 2012). Following the consumption of a mixed meal, the mobilisation of NEFAs from adipose tissue is suppressed by insulin which exerts an inhibitory effect on ATGL and HSL (Frayn et al., 1993; Frayn & Evans, 2019). As a consequence, plasma concentrations of NEFAs are highest in the fasted state (0.5 $mmol \cdot l^{-1}$) and reduced following feeding (Bickerton et al., 2007; Frayn et al., 1993).

Conversely, dietary lipids (chylomicron-derived TGs) packaged in the enterocyte and secreted into the systemic circulation increase plasma TG concentrations after a meal (Frayn & Evans, 2019; Hodson & Fielding, 2010). Unlike blood glucose, plasma TG concentrations increase over a longer time-course and peak around two to four hours postprandially in healthy individuals (Bickerton et al., 2007; Frayn et al., 1993). Following a typical mixed meal containing 20-30 g of fat, assuming an average dietary fat intake of 70-100 g per day (Frayn, 1997; 2016) across three to four eating occasions (Wittig et al., 2017), plasma TG concentrations do not increase by more than 1.0 $mmol \cdot l^{-1}$ in healthy adults (Coppack et al., 1990; Edinburgh et al., 2017). Therefore, the perturbations in systemic TG concentrations are less pronounced compared to blood glucose responses in the postprandial state (Edinburgh et al., 2017).

Adipocytes are the primary site of chylomicron-derived TG uptake and storage. Lipoprotein lipase (LPL) is bound to the endothelial cells in the dense network of capillaries surrounding adipose tissue and is up-regulated by insulin and hydrolyses chylomicron-derived TGs to allow transport across the plasma membrane into adipocytes, before re-esterification and storage as TGs in lipid droplets (Fielding & Frayn, 1998; Frayn et al., 2006). LPL is not exclusive to adipose tissue and is also expressed in skeletal muscle and the heart (Fielding & Frayn, 1998). Adipose tissue therefore acts a reservoir, mobilising and storing fatty acids under the regulation of insulin similar to the role of the liver in glucose metabolism (Frayn, 2016).

However, some NEFAs cleaved from chylomicron-derived TGs escape adipose tissue uptake and instead appear in the plasma NEFA pool, often referred to as spillover NEFAs or chylomicron-derived spillover NEFAs, and are subsequently removed by the liver (Hodson & Gunn, 2019a). The liver is also the primary tissue for chylomicron remnant uptake formed by the liberation of dietary TGs from chylomicrons by LPL (Cooper, 1997; Frayn et al., 2006). These lipids are then mixed with the existing endogenous sources in the intrahepatic fatty acid pool. In addition to suppressing lipolysis from adipose tissue, insulin secreted in the transition to the postprandial state also upregulates fatty acid synthesis from non-lipid fuels including glucose, fructose, and lactate via DNL and shifts hepatic metabolism away from oxidation and towards esterification (Hodson & Fielding, 2010; Hodson & Frayn, 2011; Hodson & Gunn, 2019a).

Concomitant to the metabolism of chylomicrons is the endogenous pathway of lipoprotein metabolism which involves the synthesis and distribution of TGs from the liver to other tissues in the form of VLDLs. Like chylomicrons, fatty acids are removed from VLDLs via LPL to allow uptake by the peripheral tissues. In the postprandial state, both chylomicrons and VLDLs compete for LPL hydrolysis following upregulation by insulin. LPL has an affinity for the larger chylomicrons and so acts on them preferentially

(Frayn & Evans, 2019). However, the removal of TGs from plasma in the postprandial period is also influenced by an individuals VLDL-TG concentration. The uptake of TGs typically occurs more quickly in those with low VLDL-TG concentrations because the competition between exogenous and endogenous TG sources for LPL causes an increase in VLDL-TGs (due to reduced clearance) so that a larger than expected rise in total plasma TG concentration occurs from the appearance of chylomicron-TGs which are then preferentially cleared (Coppack et al., 1990; Griffiths et al., 1994). Insulin also acts to suppress VLDL output in this period to avoid further increases in plasma TG concentrations. Rapid clearance, or rather the prevention of large peaks and/or prolonged exposures to exaggerated TGs appear to be beneficial because of the established associations between postprandial dysmetabolism, CVD, T2DM and NAFLD (Kolovou et al., 2019a).

The metabolism of LDLs is also an important consideration in this context. VLDLs may undergo several passes of LPL as they circulate and therefore become increasingly depleted of their TGs. These remnant particles, now LDLs following lipolysis, can either be removed by LDL receptors, including those in the liver, or remain in the circulation as cholesterol transporters. Defective, or 'leaky' sections of the endothelium caused by inflammatory cytokines from excess adipose tissue and overexpression of vascular cell adhesion molecule-1 (VCAM-1) promote blood leukocyte adherence to the vascular wall, making it permeable to potentially atherogenic lipoproteins, including LDLs, and allowing entry and retention in the intima (Falk, 2006; Libby, 2012). In the vascular wall, LDLs are oxidised by macrophages, endothelial cells, and smooth muscle cells (Yoshida & Kisugi, 2010). Macrophages scavenge and consume LDLs in an unregulated fashion, leading to the formation of cholesterol rich foam cells and the subsequent development of fatty legions, i.e. atheroma (Falk, 2006; Yoshida & Kisugi, 2010). Atherogenesis is therefore more complex than a disease of lipid storage and is reflective of ongoing

inflammation (Yoshida & Kisugi, 2010). CVD is the leading cause of mortality in those living with NAFLD (Younossi et al., 2023), in addition to sharing common risk factors of MetS, insulin resistance and T2DM (Section 2.4.7). More recent evidence suggests that LDL receptor activity may be suppressed in NAFLD, thereby reducing LDL/remnant particle clearance, and increasing the concentration of circulating atherogenic lipids (Deprince, Haas & Staels, 2020). This is further exacerbated by augmented VLDL-TG synthesis and output. In summary, intrahepatic lipid content is therefore managed by spillover NEFAs and chylomicron remnant influx and fatty acid removal either by oxidation of NEFAs or esterification and mobilisation of VLDL-TGs (Hodson et al., 2020; Hodson & Frayn, 2011). Disturbances in fatty acid input, synthesis and output subsequently perturb this balance and can result in lipid accumulation within the liver known as hepatic steatosis (Green et al., 2018; Hodson & Frayn, 2011), which is a prerequisite for the diagnosis of NAFLD (2.4.4, Clinical definition and diagnosis) (Marchesini et al., 2016; Parry & Hodson, 2017).

2.4 Non-alcoholic fatty liver disease

2.4.1 Introduction and overview

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver histology and can be categorised into non-alcohol fatty liver (NAFL) or NASH (Chalasani et al., 2012, 2018; Marchesini et al., 2016). Both NAFL and NASH are characterised by hepatic steatosis in the absence of excessive alcohol consumption, viral infection, steatogenic medications, or other secondary causes of hepatic lipid accumulation (Chalasani et al., 2012, 2018; National Guideline Centre (UK), 2016). NASH is further accompanied by inflammation and hepatocyte injury in the form of ballooning, with or without fibrosis (Chalasani et al., 2018; Marchesini et al., 2016). Fatty infiltration of the liver was traditionally viewed as a benign, non-progressive course, and commonly referred to as

‘simple’ or ‘bland’ steatosis (Dam-Larsen et al., 2004; Teli et al., 1995b). Conversely, NASH was considered the progressive form of the disease with increased liver-related morbidity and mortality (De & Duseja, 2020). It is now recognised that NAFLD is a dynamic condition with patients demonstrating varying degrees of progression and regression (De & Duseja, 2020; Reddy et al., 2020). Instead, the presence and stage of hepatic fibrosis, representing the wound healing response to liver injury (Jiao et al., 2009), appears to be a key prognostic predictor of liver-related and all-cause mortality (Angulo et al., 2015; Taylor et al., 2020a). Liver fibrosis can also progress in both NAFL and NASH (Singh et al., 2015). In a small number of patients, NAFLD may proceed further to cirrhosis, liver cancer and the need for transplantation (Marchesini et al., 2016; National Guideline Centre (UK), 2016).

Although hepatic steatosis has been recognised at autopsy since the 1800s, it was typically considered to be the manifestation of alcoholic liver disease (ALD) or alcoholic steatohepatitis (ASH) (Torbenson & Washington, 2020). Patients with biopsy specimens characteristic of assumed ASH, yet who denied excessive alcohol intake, were subject to undue perseverance from clinicians in an attempt to ‘wrench’ from them an admission of excessive alcohol consumption or to obtain confirmation of such habits from the patients relatives (Ludwig et al., 1980). In the late 1970s, Adler and Schaffner reported liver function and biopsy findings in 29 overweight patients who, following individual interviews with separate clinicians, were considered light social drinkers (Adler & Schaffner, 1979). Histological features of hepatic lipid infiltration were observed with equal distribution across the categories of ‘fatty liver’, ‘fatty hepatitis’, ‘fatty fibrosis’, and ‘fatty cirrhosis’. Diabetes and dyslipidaemia were also observed across these histologically defined groups (Adler & Schaffner, 1979). The authors concluded that the pathological findings were a common denominator of both obesity and alcohol induced liver disease (Adler & Schaffner, 1979). Following similar findings, the term NASH was

subsequently conceived by Ludwig and colleagues a year later to describe this ‘hitherto unnamed disease’ (Ludwig et al., 1980).

The emergence of NAFLD over recent decades has paralleled that of the coexisting obesity epidemic (Dietrich & Hellerbrand, 2014; Koppe, 2014). NAFLD is associated with insulin resistance and a clustering of metabolic risk factors, including: hypertension, hyperglycaemia and dyslipidaemia (Armstrong et al., 2014; Younossi et al., 2016). Unsurprisingly, NAFLD is considered the hepatic manifestation of MetS and the subsequent development of CVD and T2DM (Anstee et al., 2013; Younossi et al., 2016). Initially thought of as a disease of the affluent West (Day, 2006), the estimated global prevalence of NAFLD is 30% with the highest number of cases Latin America and the Middle East (Younossi et al., 2023). NAFLD is recognised as the primary cause of chronic liver disease worldwide and is predicted to be the leading indication for liver transplantation (Estes et al., 2018; Pais et al., 2016). Therefore, NAFLD poses a significant health and economic burden to all societies (National Guideline Centre (UK), 2016; Younossi et al., 2016).

2.4.2 Pathogenesis of NAFLD

The pathogenesis of NAFLD is multifaceted and involves complex interactions between genetic, nutritional and environment factors (Arab et al., 2018; Hardy et al., 2016). A thorough appraisal of these topics is therefore outside the scope of the current review and is available elsewhere (Grander et al., 2023; Khairnar et al., 2023; Manne et al., 2018). Instead, the discussion to follow will focus on the metabolic and endocrine perturbations involved in the development of the disease.

Hepatic fatty acid infiltration is the unequivocal defining feature of NAFLD (Chalasani et al., 2018; Marchesini et al., 2016). As described above, hepatic steatosis represents an imbalance between fatty acid uptake, storage and utilisation, as determined by: a)

postabsorptive and postprandial adipose tissue lipolysis, b) hepatic uptake of dietary fatty acids, c) synthesis and storage of TGs in hepatic lipid droplets, d) hepatic DNL of non-lipid precursors, e) oxidation of hepatic fatty acids, and f) packaging and mobilisation of TGs as VLDL-TGs (Hodson et al., 2020; Hodson & Frayn, 2011). Disordered lipid metabolism is therefore central to the pathogenesis of NAFLD.

2.4.3 Natural history of NAFLD

Knowledge regarding the natural history of NAFLD is evolving, however it is not yet fully understood (De & Duseja, 2020; Lindenmeyer & McCullough, 2018). As highlighted above, early follow-up studies suggested that simple steatosis was a benign, non-progressive condition that did not increase liver-related or all-cause mortality (Dam-Larsen et al., 2004; Teli et al., 1995b). Instead, it was thought that NASH was the progressive form of the disease with increased liver-related deaths because of cirrhosis and liver cancer (De & Duseja, 2020; Ekstedt et al., 2006). The traditional view that fatty live disease (FLD) has a more favourable prognosis than NASH, coupled with the relatively limited number and length of longitudinal follow-up studies may, at least in part, explain why our understanding remains incomplete (McPherson et al., 2015).

However, several studies have provided data to challenge this dogma (Angulo et al., 2015; McPherson et al., 2015; Reddy et al., 2020). For example, McPherson and colleagues (2015) followed 108 patients over a median follow-up of 6.6 years of which 75% had NASH and the remainder had FLD. Whilst fibrosis progressed in approximately 40% of patients, it remained unchanged (40%) or regressed (~ 20%) in the rest of the cohort (McPherson et al., 2015). Furthermore, progression to NASH was observed in 44% of patients diagnosed with FLD at baseline. These data therefore contradict the long-held belief that few patients with hepatic steatosis subsequently develop NASH. The authors also reported a significantly higher prevalence of T2DM at both baseline and follow-up

in patients with fibrotic progression (McPherson et al., 2015). At baseline, 21% of patients with FLD had T2DM compared to 56% of those with NASH. At follow-up, among patients with FLD, 80% of those with fibrosis progression had T2DM, in contrast to 25% of patients with no progression. The presence or development of T2DM therefore appears to be a significant risk factor for fibrotic progression (McPherson et al., 2015; Reddy et al., 2020). Moreover, the presence and grade of fibrosis, independent of NASH, is the greatest prognostic indicator for liver-related complications, liver-transplantation, and overall mortality (Angulo et al., 2015; Ekstedt et al., 2015). In a retrospective study, Adams and associates (2005) observed that 12.6% of 420 patients with NAFLD died over a median follow-up of 7.6 years; a significantly greater mortality rate to that expected for the general population matched for age and sex (Adams et al., 2005). Liver-related and all-cause mortality is further increased in those with NASH compared to FLD and to the general population (Ekstedt et al., 2006; Söderberg et al., 2010; Younossi et al., 2016). However, when adjusted for fibrosis stage, the presence of NASH does not increase the risk of liver-specific morbidity or overall mortality; further emphasising the prognostic importance of fibrosis in the absence of all other histological features (Hagström et al., 2017).

Despite these recent insights, there are comparatively limited data on the time-course and long-term outcomes of advanced fibrosis and cirrhosis owing to NAFLD (Lindenmeyer & McCullough, 2018; Vilar-Gomez et al., 2018). The world-wide incidence of advanced fibrosis in NASH has been estimated to be 67.95 in 1000 person-years, with 41% of patients with NASH demonstrating fibrosis progression (Lindenmeyer & McCullough, 2018; Younossi et al., 2016). Up to a quarter of patients with NAFLD will progress to cirrhosis (McCullough, 2004; Önnerhag et al., 2014) and 7% to end-stage liver disease (Ekstedt et al., 2006). Vilar-Gomez and co-workers (2018) documented that patients with cirrhosis (F4) were more likely than those with bridging fibrosis (F3) to have hepatic

decompensation or liver cancer; concluding that cirrhosis resulted in predominantly liver-related events, whereas those with bridging fibrosis primarily developed non-hepatic cancers and vascular events (Vilar-Gomez et al., 2018).

Although it is now evident that NAFLD is dynamic in nature, further prospective longitudinal studies are needed to elucidate the natural history of the disease, particularly in patients at the advanced end of the pathological spectrum (Ekstedt et al., 2017). It is hoped that further understanding of the natural history, with the identification of factors associated with progression and long-term outcomes, will result in improved prevention, screening, monitoring and treatment modalities (Lindenmeyer & McCullough, 2018). The interested reader is directed to several narrative reviews (De & Duseja, 2020; Ekstedt et al., 2017; Lindenmeyer & McCullough, 2018) and systematic reviews with meta-analyses (Singh et al., 2015; Taylor et al., 2020a) for a more comprehensive discussion of the natural history of NAFLD.

2.4.4 Clinical definition and diagnosis

Until recently, NAFLD was defined as the presence of steatosis, confirmed by either histology or imaging, and the exclusion of secondary causes of fat accumulation, including a daily alcohol intake of ≤ 30 g for men and ≤ 20 g for women (Chalasani et al., 2018; Marchesini et al., 2016). Evidence of steatosis in > 5% of hepatocytes as determined by histology or by a magnetic resonance imaging proton density fat fraction (MRI-PDFF), or > 5.6% assessed by proton magnetic resonance spectroscopy (^{1}H-MRS) or quantitative fat/water selective MRI (Marchesini et al., 2016).

However, proposed changes in the nomenclature have seen a transition from NAFLD to metabolic dysfunction-associated fatty liver disease (MAFLD, Figure 2.3) (Eslam et al., 2020a, 2020b) to metabolic dysfunction-associated steatotic liver disease (MASLD) (Rinella et al., 2023). The latter now sits under the overarching term of steatotic liver

disease (SLD) (Figure 2.4). The rationale for these changes appears to be two-fold. Firstly, to reflect the increasing recognition of metabolic dysfunction, including many features of MetS, that are frequently observed in those individuals living with lifestyle induced liver disease (Rinella et al., 2023). Secondly, to remove the perceived stigma associated with the terms 'non-alcoholic' and 'fatty' (Rinella et al., 2023). It is proposed that these updated diagnostic criteria and nomenclature will improve awareness and patient identification. A growing number of studies have subsequently sought to compare NAFLD, MAFLD and MASLD diagnostic criteria to determine the prevalence of each within the same cohort. For example, Song and colleagues (2024) found the prevalence of NAFLD, MAFLD and MASLD was 18.5%, 19.3% and 20.8%, respectively, in a sample from the National Health and Nutrition Examination Surveys in the United States. Those individuals with NAFLD (94.5%) or MAFLD (100%) were also classified as MASLD. However, a lower proportion of those with MASLD were similarly diagnosed with NAFLD (84.1%) or MAFLD (92.7%) (Song et al., 2024). In contrast, the prevalence of NAFLD, MAFLD and MASLD were similar (34.7%, 34.9% and 33.4%, respectively) in a cohort from the Brazilian Longitudinal Study of Adult Health (Perazzo et al., 2024).

With reference to lifespan, both MAFLD and MASLD but not NAFLD were associated with higher all-cause mortality during a median follow-up of 26.9 years (Song et al., 2024). However, all three of the nomenclature were associated with an increased risk of all-cause mortality in those with advanced fibrosis. These data highlight the complexities of developing encompassing terms for what is a multifaceted and dynamic disease; as well as the need for further refinement as our knowledge and understanding continue to advance (Ramírez-Mejía & Méndez-Sánchez, 2023).

For the purpose of the current thesis the terminology and definition of NAFLD (Chalasani et al., 2018; Marchesini et al., 2016), opposed to MAFLD (Eslam et al., 2020a, 2020b) or MASLD (Rinella et al., 2023) will be utilised. This decision is based on the wealth of

literature available pertaining to the well-established concept of NAFLD and the proposed evaluation of associations between lifestyle factors, including alcohol and MetS characteristics (Chapter 4). This approach may provide evidence to support the over-reaching concept of MASLD; using blood biomarkers and associated metabolic risk abnormalities.

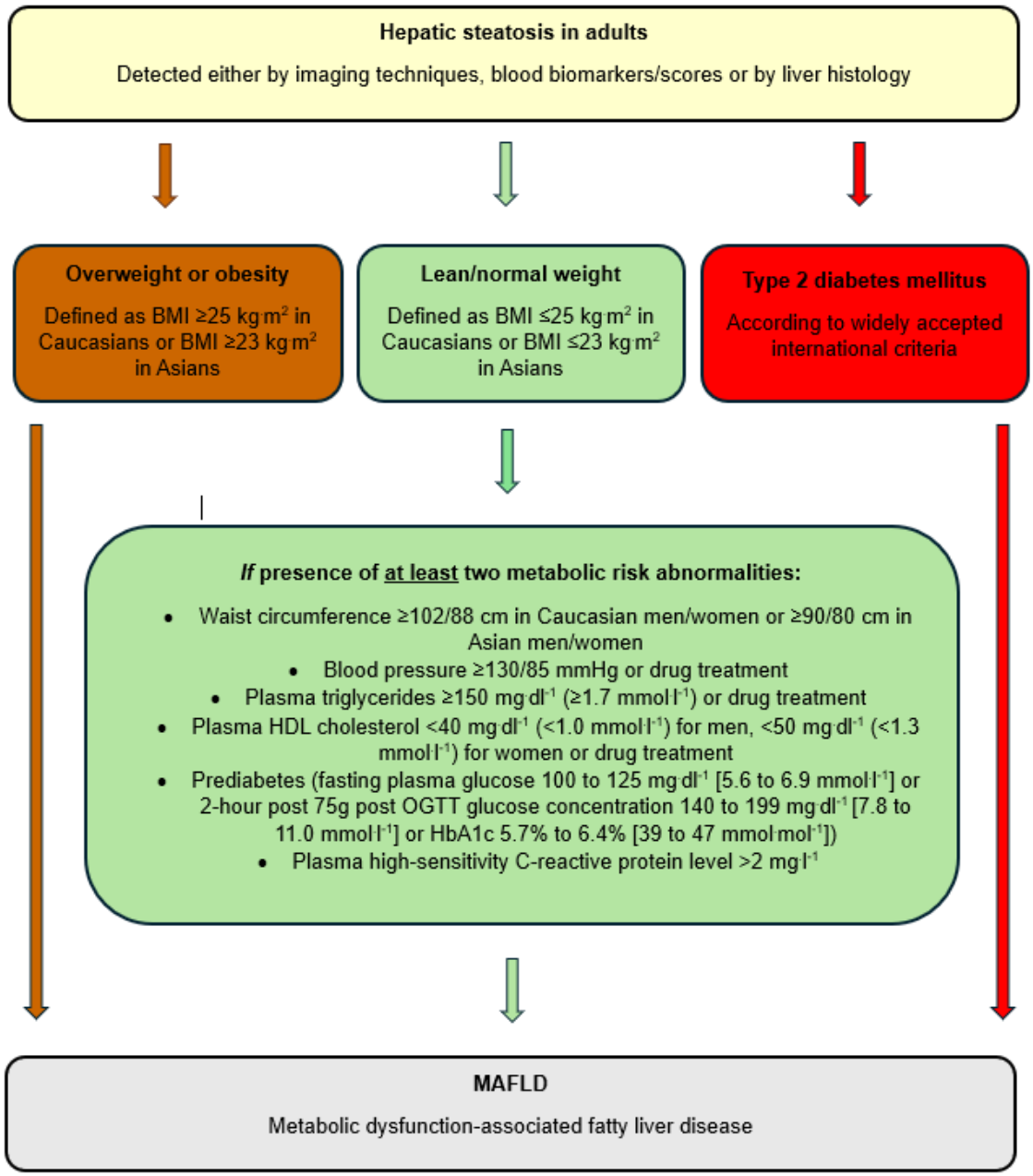

Figure 2.3 Proposed diagnostic criteria for metabolic associated fatty liver disease (MAFLD). Adopted from Eslam et al. (2020a).

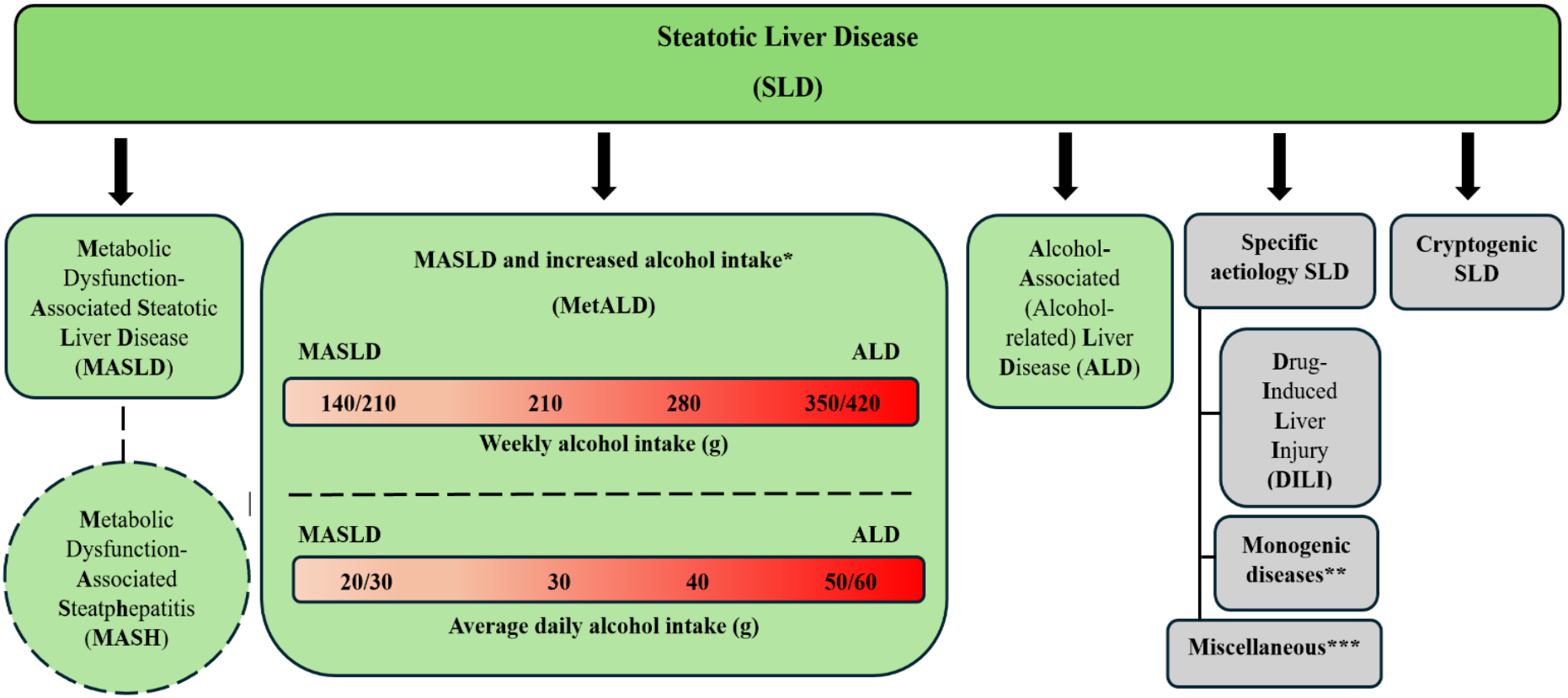

Figure 2.4 Steatotic liver disease (SLD) subclassification. *Weekly intake 140-350 g for females and 210-420 g for males (average daily intake 20-50 g for females and 30-60 g for males). **e.g., Lysosomal acid lipase deficiency (LALD), Wilson disease, hypobetalipoproteinaemia, inborn errors of metabolism. ***e.g., malnutrition, celiac disease, human immunodeficiency virus (HIV). Adopted from Rinella et al. (2023).

2.4.5 Diagnostic modalities

Hepatic steatosis is the defining histological feature of NAFLD and can be determined utilising both invasive and non-invasive methodologies, including liver biopsy and several imaging techniques (Chalasani et al., 2018; Marchesini et al., 2016). A number of minimally invasive serum markers and composite scoring systems have also been developed as proxy indicators of hepatic fat accumulation and fibrosis staging (Castera et al., 2019). Briefly, the discussion to follow will consider liver biopsy and imaging techniques before appraising the merits and limitations of surrogate panels utilised in Chapter 4 of the current thesis. The interested reader is directed to narrative reviews by Castera et al. (2019), Tsai & Lee, (2018) and Younossi et al. (2018) for comprehensive overviews of techniques for the identification and staging of NAFLD.

2.4.5.1 Liver biopsy

The direct measurement of hepatic fatty infiltration and fibrosis by liver biopsy remains the 'gold standard' method for diagnosing NAFLD and differentiating between NAFL and NASH (Chalasani et al., 2018; Marchesini et al., 2016). This is despite several limitations, for example, high cost, sampling error, poor patient acceptability, and potential morbidity and mortality risks (Chalasani et al., 2018; Marchesini et al., 2016). These caveats preclude repeated sampling and therefore limit the utility of liver biopsy as a tool to monitoring histological changes long-term (Xiao et al., 2017). This is further demonstrated by the disparity between published guidance and the use of liver biopsy in clinical practice, with approximately a quarter of gastroenterologists and hepatologists routinely performing liver biopsies in patients with presumed NASH (Rinella et al., 2016). Similarly, given the prevalence of NAFLD, it is not feasible or practical to perform liver biopsy in all patients (Jayakumar et al., 2016; Whalley et al., 2007). The use of liver

biopsy as the reference standard has also precluded the population-based study of NASH, to which limited epidemiological data are available (Younossi et al., 2016).

It is therefore recommended that liver biopsy is utilised in those who would benefit the most from diagnosis, therapeutic, and prognostic information, including patients with MetS and those with co-existing chronic liver disease (Chalasani et al., 2012, 2018). A more thorough commentary regarding the role of histology in clinical research and the management of NAFLD patients has been provided by Younossi and colleagues (2018).

2.4.5.2 Imaging techniques for detecting and quantifying steatosis

As a consequence of the limitations of liver biopsy, there has been significant interest in alternative non-invasive techniques to accurately quantify hepatic fat content (Castera, 2015; Castera et al., 2019). Although several imaging techniques have been developed and reviewed by others (Castera et al., 2019; Younossi et al., 2018), this discussion will focus on those methods included in the definition and diagnosis of NAFLD and used routinely in the standard care pathway, specifically, ^{1}H-MRS and MRS, and ultrasound (US) (Chalasani et al., 2018; National Guideline Centre (UK), 2016).

The utility of non-invasive techniques is determined using the receiver operator characteristic (ROC) curve by comparing non-invasive methods to a reference standard, such as liver biopsy. ROC analysis is a statistical procedure used to characterise a tests diagnostic accuracy, i.e., the ability to correctly dichotomise those individuals with versus without a particular condition (Obuchowski & Bullen, 2018). Basic metrics of accuracy include sensitivity and specificity. Sensitivity is estimated as the number of patients with a particular condition who were correctly identified (true positives) divided by the total number of patients with the condition (true positives and false negatives). Specificity is estimated as those without a particular condition who were corrected identified (true negatives) divided by the total number of patients without the condition (true negatives

plus false positives) (Obuchowski & Bullen, 2018). The ROC curve represents a measure of a diagnostic tests sensitivity plotted on the y-axis versus its false positive rate (those without the condition identified as positive divided by the total number of patients without the condition) on the x-axis. The area under the ROC (AUROC) curve is the most commonly used summary statistic because of its simplicity (Obuchowski & Bullen, 2018). A diagnostic test that discriminates perfectly between two conditions will have an AUROC curve of 1.0, whilst the AUROC curve of a test with no diagnostic ability will be 0.5 (Obuchowski & Bullen, 2018).

Other metrics of diagnostic accuracy are the positive predictive value (PPV) and the negative predictive value (NPV). These statistics are similarly determined from the construction of a 2 x 2 table (i.e., true positive, false negative, false positive and true negative). The PPV is calculated as the number of true positives divided by the total number of cases identified as positive (true positives plus false positives). The PPV is therefore the probability that patients with a positive screening result indeed have a particular condition (Trevethan, 2017). The NPV is calculated as the number of true negatives divided by the total number of cases identified as negative (false negatives and true negatives), and therefore represents the probability that patients with a negative screening result indeed do not have a particular condition (Trevethan, 2017). These metrics are important for instances in which the correct diagnosis could allow for early intervention or used to guide decisions regarding treatment and so prevent disease progression. Or, conversely, where the incorrect diagnosis could result in a lack of intervention or delayed treatment potentially causing significant harm (Trevethan, 2017). For example, the early identification of hepatic fibrosis and the subsequent monitoring and staging of disease progression. In this scenario, a high NPV is desirable for ruling out advanced fibrosis (Castera et al., 2019; Trevethan, 2017).

Conventional US is the most commonly used imaging technique for the initial diagnosis of hepatic steatosis and is preferred because it is widely available, well tolerated by patients, and inexpensive (Castera et al., 2019; Stern & Castera, 2017). It allows for the indirect estimation of fatty infiltration based on subjective qualitative features such as hyperechogenicity, making the liver appear brighter than adjacent splanchnic structures (Castera et al., 2019; Stern & Castera, 2017). Liver fat accumulation can be determined qualitatively as 'absent', 'mild', 'moderate', or 'severe' or semi-quantitatively using ordinal scores (Castera et al., 2019; Ferraioli & Soares Monteiro, 2019).

A meta-analysis that included 49 studies demonstrated that US provides an accurate diagnosis of moderate-to-severe steatosis, defined as histological grade 20%-33%, with an AUROC curve of 0.93 (Hernaez et al., 2011; Stern & Castera, 2017). However, the presence of 5%-10% hepatic fatty infiltration is considered abnormal, with > 5% steatosis characteristic of NAFLD (Chalasani et al., 2018; Younossi et al., 2018). A significant number of patients with mild steatosis (≥ 3%-5% histological grade) may therefore evade diagnosis (Castera et al., 2019). Indeed, the sensitivity and specificity of US varies widely and has been reported to range from 53% to 100%, and from 77% to 98%, respectively (Esterson & Grimaldi, 2018). Accordingly, higher sensitivity and specificity values are observed for moderate-to-severe steatosis and lower values reported when all grades of fatty infiltration are taken as a collective, including mild steatosis (Esterson & Grimaldi, 2018). Additional pitfalls include reduced specificity in the presence of co-existing chronic liver disease, and inter- and intra-operator variability (Esterson & Grimaldi, 2018; Green et al., 2018). Moreover, the diagnostic performance of US is further limited in obesity (Bril et al., 2015), which is an almost universal presentation and tightly associated with NAFLD. The disparate diagnostic accuracy of US for the quantification of steatosis is therefore perhaps not surprising. More recently, contemporary studies have documented improved diagnostic accuracy when utilising modified quantitative US

methods (Castera et al., 2019; Esterson & Grimaldi, 2018). Nevertheless, despite its limitations, US continues to be recommended as the preferred first choice imaging mode for adults at risk of NAFLD in Europe (Marchesini et al., 2016; National Guideline Centre (UK), 2016).

In contrast, MRI techniques are able to accurately detect small quantities of steatosis and are considered the 'gold standard' imaging modality for the assessment of hepatic fatty infiltration (Esterson & Grimaldi, 2018; Stern & Castera, 2017). Briefly, ^{1}H-MRS detects spectral peaks that are characteristic of the multiple hydrocarbon components within fat and therefore has a distinctive chemical signature (Esterson & Grimaldi, 2018; Younossi et al., 2018). MRI PDFF determines the fraction of protons bound to fat in the liver divided by the total number of protons in the liver, bound to both fat and water. The alignment of these protons and the contrast in the strength of the signal at known echo times allows for the determination of hepatic fat content (Esterson & Grimaldi, 2018; Green et al., 2018). Both ^{1}H-MRS and MRI-PDFF are able to detect > 5.6% steatosis, the diagnostic threshold for NAFLD, with close to 100% accuracy (Green et al., 2018; Reeder et al., 2011). Moreover, compared to US, MRI techniques have demonstrated significantly greater accuracy for the determination of hepatic fatty acid content and, crucially, can be used prospectively for patient follow-up (Lee et al., 2010; van Werven et al., 2010). However, ^{1}H-MRS and MRI-PDFF are not without their limitations, including high cost and the need for technical expertise. In addition, MRI techniques are less accessible and typically reserved for the research setting (Castera et al., 2019; Stern & Castera, 2017).

2.4.5.3 Imaging techniques for detecting and staging fibrosis

As aforementioned, the presence and stage of hepatic fibrosis is a key prognostic indicator of liver-related and all-cause mortality (Angulo et al., 2015; Taylor et al., 2020). Methods

for the early identification and accurate staging of fibrosis are therefore required to monitor disease progression. Similar to the quantification of steatosis, liver biopsy, despite its limitations, remains the 'gold standard' method for the detection and staging of fibrosis (Chalasani et al., 2018; National Guideline Centre (UK), 2016). Using the Metavir scoring system, fibrosis is staged on an ordinal scale defined as: F0, no fibrosis; F1 portal fibrosis without septa; F2, portal fibrosis with few septa extending outside of portal areas; F3, bridging fibrosis or numerous septa without cirrhosis; and F4, cirrhosis (Goodman, 2007; Tsai & Lee, 2018). Other fibrosis scoring systems with a similar F0 to F4 ordinal scales are also utilised, including Brunt and Kleiner (Kleiner et al., 2005), Ludwig (Ludwig et al., 1978) and the steatosis, activity and fibrosis (SAF) score (Bedossa et al., 2014). Imaging techniques, including conventional US and MRI, have been adapted to evaluate liver stiffness or 'elasticity' as a surrogate parameter for fibrosis (Esterson & Grimaldi, 2018; Younossi et al., 2018). In elastography, an external force is applied to the liver that momentarily distorts the hepatic parenchyma and produces shear waves which propagate through the liver perpendicular to the direction of the force (Esterson & Grimaldi, 2018). Imaging modalities, including ultrasound-based acoustic radiation force impulse imaging (ARFI) and magnetic resonance elastography (MRE), are able to measure the velocity of these shear waves and quantify the degree of stiffness and so fibrosis (Esterson & Grimaldi, 2018).

Cui and colleagues (2016) compared the two methodologies to biopsy-proven fibrosis in NAFLD patients and observed that MRE demonstrated superior accuracy for the determination of all fibrosis stages (≥ F1), both in the overall study cohort and in obese participants (AUROC curve of 0.80 and 0.85, and 0.66 and 0.60, for MRE and ARFI, respectively) (Cui et al., 2016). The use of MRE therefore overcomes the limitations of AFRI, including the technical challenges involved in those living with obesity and significant ascites (Dulai et al., 2016; Younossi et al., 2018). MRE is however not without

its pitfalls, many similar to those of other MRI techniques, including: expense, non-routine availability, and the need for technical expertise (Esterson & Grimaldi, 2018; Younossi et al., 2018).

2.4.5.4 Composite panels for identifying steatosis

The limitations of liver biopsy and sophisticated imaging techniques negate their routine use and are instead reserved for the secondary care and research settings. A number of minimally invasive composite panels to predict the presence and grading of steatosis or fibrosis have subsequently been developed. These surrogate panels typically combine parameters from routine serology, including liver enzymes; blood glucose; and TGs; and anthropometric measurements such as BMI and WC (Bedogni et al., 2006; Harrison et al., 2008). Composite panels therefore have several practical advantages, for example, little if any additional costs, easy to calculate, good inter-laboratory reproducibility and the potential for widespread use (non-patented); making them ideal for use in primary care and population-based studies (Adams et al., 2011; Castera et al., 2019).

Several proxy scores have been developed to identify steatosis, including the FLI (Bedogni et al., 2006), lipid accumulation product (LAP) (Kahn, 2005), Steatotest (Poynard et al., 2005), Hepatic Steatosis Index (Lee et al., 2010), and the NAFLD Liver Fat Score (Kotronen et al., 2009). Pertinent to the thread of the current thesis are the FLI and LAP. Briefly, the FLI algorithm is comprised of fasting TGs, GGT, BMI and WC to generate a score between 0 and 100 (Bedogni et al., 2006). Similarly, the LAP also utilises fasting TGs and WC; however, has sex specific calculations to generate a continuous score (Kahn, 2005).

The diagnostic accuracy of both FLI and LAP were originally determined against US as the reference standard; with an AUROC curve of 0.84 and 0.79, respectively (Bedogni et al., 2006, 2010; Cuthbertson et al., 2014). The diagnostic performances of FLI and LAP

are approximate to that of other composite panels highlighted above, with AUROC curves ranging between 0.77 and 0.87 (Stern & Castera, 2017). However, to directly compare the accuracy of each proxy score is challenging because of the validation against different reference standards; including US, ^{1}H-MRS, and liver biopsy (Stern & Castera, 2017). Both FLI and LAP have been externally validated against ^{1}H-MRS in a cohort of healthy controls and obese, insulin resistant individuals (Cuthbertson et al., 2014). The FLI and LAP again performed favourably with AUROC curves of 0.79 and 0.78 for identifying steatosis. The FLI and LAP may therefore be utilised to identify patients with hepatic steatosis in the clinical setting or for research purposes (Cuthbertson et al., 2014).

2.4.5.5 Composite panels for identifying and staging fibrosis

A number of minimally-invasive composite panels have also been developed for the identification and staging of hepatic fibrosis (Tsai & Lee, 2018; Xiao et al., 2017). These range from simple to more complex models, including: the aspartame aminotransferase to alanine aminotransferase ratio (AST:ALT) (Williams & Hoofnagle, 1988); alanine to platelet ratio index (APRI) (Wai et al., 2003); the BMI, AST:ALT ratio and diabetes (BARD) score (Harrison et al., 2008); fibrosis 4 (FIB-4) (Sterling et al., 2006); and the NAFLD fibrosis score (NFS) (Angulo et al., 2007). Several studies have compared the diagnostic accuracy of these panels (Adams et al., 2011; Sun et al., 2016; Xiao et al., 2017). Xiao and colleagues (2017) reported AUROC curves of 0.76, 0.77, 0.84 and 0.84 for the BARD score, APRI, FIB-4 and NFS, respectively, in diagnosing advanced fibrosis (F3-F4) with liver biopsy as the reference standard (Xiao et al., 2017). Separately, Shah and co-authors (2009) documented an AUROC curve of 0.74 for the AST:ALT for the detection of advanced fibrosis when again utilising liver histology as the reference (Shah et al., 2009). These observations are consentient with others who have also reported greater diagnostic accuracy with complex compared to simple models for predicting fibrosis stage (Adams et al., 2011; Sun et al., 2016). Furthermore, the increasingly

complex panels also demonstrated the greatest sensitivity and specificity with summary values of 75% and 61%, and 73% and 74%, for the BARD score and NFS with cut-off values of ≥ 2 and > 0.676 for advanced fibrosis, respectively (Xiao et al., 2017).

As previously discussed, the NPV is an important metric when misdiagnosis and the resulting delay or absence of intervention has the potential to cause harm (Trevethan, 2017). Of the four composite panels included in their analysis, Xiao and co-workers (2017) reported that the FIB-4 and NFS had the highest NPV (> 90%) for ruling out advanced fibrosis. Potentially, these panels could therefore be utilised in the primary care setting to identify patients without advanced fibrosis and avoid unnecessary further assessment or referral to secondary care (Castera et al., 2019). Nevertheless, there are also pitfalls to the NFS and FIB-4 that should be noted. Despite the high NPV, both models have limited utility to predict (rule in) advanced fibrosis with PPVs of 50.4% and 40.3% for the NFS and FIB-4, respectively; meaning that an alternative assessment may be needed to confirm positive results (Castera et al., 2019; Xiao et al., 2017). Similarly, a large proportion of patients (approximately 30%) fall into the intermediate-risk category which may necessitate further investigation and/or referral to specialist care to confirm a diagnosis (Alexander et al., 2018; Castera et al., 2019). Others have however attempted to improve the diagnostic performance of the NFS and FIB-4 with new age-adjusted cut-offs for advanced fibrosis (Castera et al., 2019; McPherson et al., 2017). More recently, it has also been shown that modified cut-offs could further improve the diagnostic accuracy of minimally-invasive models in patients with morbid obesity (Meneses et al., 2020).

2.4.6 NAFLD prevalence and incidence

The worldwide prevalence of NAFLD is estimated to be 30% in the general population (Younossi et al., 2023). However, the number of cases varies across the globe, ranging from the highest in Latin America (44.37%) and the Middle East and North Africa

(36.53%) to the lowest in Western Europe (25.10%). In the UK, it is estimated that 24% of the population are living with NAFLD, a number comparable to that of other European countries (Younossi, 2019; Younossi et al., 2016, 2023). In addition to regional differences, the prevalence of NAFLD also varies by age, sex, and ethnicity (Younossi, 2019). Unsurprisingly, as age increases so does the number of NAFLD cases (Younossi et al., 2016; Younossi, 2019). NAFLD is also more prevalent in men than women (Browning et al., 2004; Marjot et al., 2020). Furthermore, there appears to be sex-specific differences in prevalence with age, with NAFLD increasing significantly after 50 years of age in women (Bedogni et al., 2010; Marjot et al., 2020). This observation may be attributed, at least in part, to the menopause, with up to double the number of NAFLD cases reported in post- compared to premenopausal women (Florentino et al., 2013; Marjot et al., 2020). Regarding ethnicity, previous studies have shown that those of Hispanic decent have a higher, and African-Americans a lower, prevalence of NAFLD compared to Caucasians (Marjot et al., 2020; Younossi, 2019). However, the reasons for these observations are complex; with genetic and environmental factors, socioeconomic status, and healthcare access all contributory factors (Marjot et al., 2020; Younossi et al., 2018).

The prevalence of NAFLD is further increased in high-risk populations such as those living with obesity and T2DM. Previous studies have reported that 67% of those considered overweight (BMI ≥ 25.0-29.9 $kg{\cdot}m^{-2}$) and 91% classified as obese (BMI ≥ 30.0 $kg{\cdot}m^{-2}$) had NAFLD as determined by US (Bedogni et al., 2007; Marjot et al., 2020). Similar observations were made in morbidly obese patients undergoing bariatric surgery, with 91% having biopsy proven NAFLD (Machado et al., 2006; Marjot et al., 2020). It is estimated that 55% of those with T2DM also have NAFLD (Younossi et al., 2019). A recent global meta-analysis, the most comprehensive to date, reported that obesity and T2DM were present in 51% and 22.5% of those with NAFLD, respectively (Younossi et

al., 2016). These data demonstrate the frequent co-existence of NAFLD, obesity and T2DM. In contrast to the well documented global prevalence of NAFLD, only limited data are available regarding incidence. Estimates range from 28.01 cases per 1,000 person-years to 52.34 cases per 1000 person-years (Younossi, 2019; Younossi et al., 2016). For comprehensive reviews of the epidemiology of NAFLD, see Younossi and colleagues (2016; 2023).

2.4.7 Metabolic health and NAFLD

Metabolic health is an umbrella term which can be defined as the ability to maintain homeostasis in response to challenging stimuli, including nutrition (Hengist et al., 2019). Metabolic health is characterised by the ability to maintain blood glucose and TG concentrations within a range that does not increase disease risk (Edinburgh et al., 2017; Hengist et al., 2019). Alternatively, metabolic health can be defined as the absence of risk factors, including hyperglycaemia and hypertriglyceridaemia (Alberti et al., 2009). MetS is a clustering of risk factors, including elevated WC, hypertension, and low serum HDL, in addition to hyperglycaemia and hypertriglyceridaemia (Alberti et al., 2009). The coexistence of three or more of these risk factors is required for the diagnosis of MetS (Alberti et al., 2009). MetS is subsequently associated with obesity (Nguyen et al., 2008), CVD (Gami et al., 2007), T2DM (Ford et al., 2008) and NAFLD (Younossi et al., 2016). As discussed above (section 2.4.4 Clinical definition and diagnosis) the influence of metabolic dysfunction in the development of NAFLD has been acknowledged more recently with a recommended shift in the nomenclature from NAFLD to MAFLD and now MASLD (Eslam et al., 2020a; Fouad et al., 2020; Younossi et al., 2023).

From a metabolic perspective, the accumulation of IHTG, the hallmark of NAFLD, appears to be primarily driven by adiposity and insulin resistance (Dearlove & Hodson, 2022; Nagarajan et al., 2022). For example, Smith and colleagues (2020) observed a

stepwise increase in fasting and postprandial insulin concentrations during an OGTT when comparing lean, obese, and obese individuals with NAFLD. The authors concluded that this was due to an increase in insulin secretion and saturation in hepatic insulin extraction; elevating circulating insulin concentrations (Dearlove & Hodson, 2022; Smith et al., 2020a, Figure 2.5). Obesity is associated with adipose tissue and skeletal muscle insulin resistance (Ahmed et al., 2021; Lee et al., 2022), and may therefore increase substrate flux to the liver, including; NEFAs, TG-rich chylomicron remnants, as well as glucose (Dearlove & Hodson, 2022; Luukkonen et al., 2022; Nagarajan et al., 2022). Indeed, peripheral insulin resistance increases hepatic DNL (Flannery et al., 2012; Rabøl et al., 2011). Within the liver, the contribution of DNL to IHTG-palmitate has been shown to be higher in obese individuals with NAFLD (~ 38%) compared to obese (~ 19%), and lean individuals (~ 11%) (Dearlove & Hodson, 2022; Smith et al., 2020b). Hepatic DNL was inversely associated with both hepatic and whole-body insulin sensitivity, however, positively correlated with 24 hour glucose and insulin measurements (Smith et al., 2020b). These investigations were repeated in a small subgroup analysis of obese-NAFLD participants following diet-induced weight-loss of approximately 10%. The contribution of hepatic DNL to IHTG-palmitate was reduced by ~ 35% and IHTG content by ~ 50%. Weight loss also improved hepatic and whole-body insulin sensitivity and attenuated 24 hour glucose and insulin responses (Smith et al., 2020b). Despite this evidence, it must be acknowledged that the relationship between obesity, insulin resistance and NAFLD could be bi-directional, i.e., steatosis may precede or be caused by obesity-associated insulin resistance (Dearlove & Hodson, 2022; Farese et al., 2012). A schematic representation of the proposed mechanisms by which obesity and insulin resistance lead to IHTG accumulation is shown in Figure 2.5. The reader is also directed to contemporary narrative reviews by Dearlove and Hodson (2022) and Nagarajan and colleagues (2022).

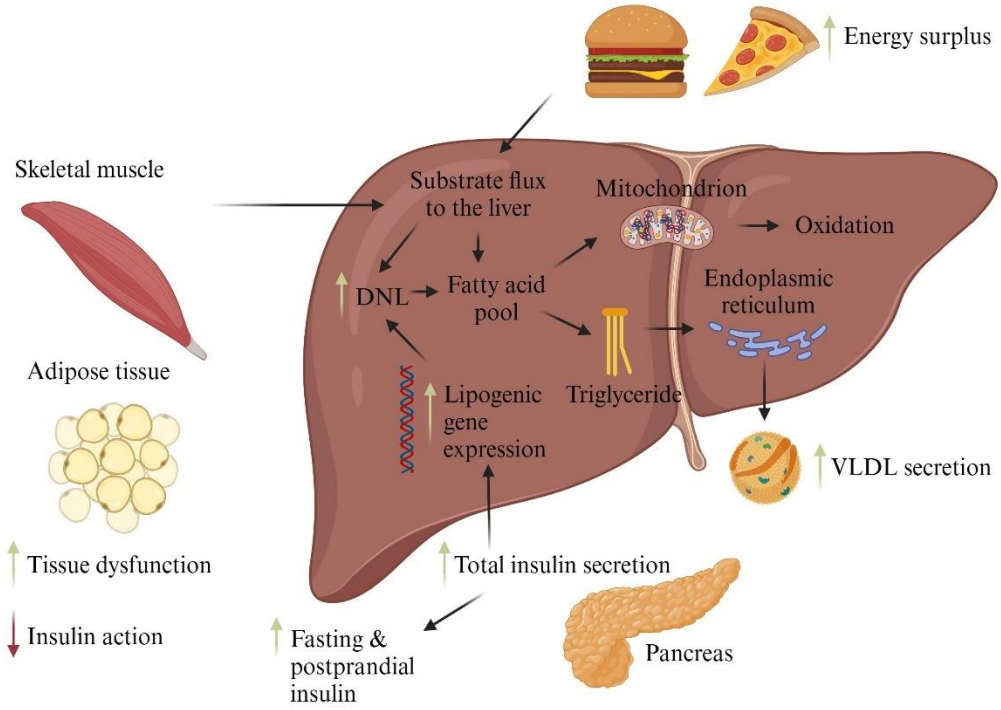

Figure 2.5 Overview of proposed mechanisms by which obesity and insulin resistance lead to IHTG accumulation. Total insulin secretion is increased in obese compared to lean individuals. However, hepatic insulin extraction may become saturated, leading to elevated circulating insulin levels. Decreased extrahepatic tissue insulin sensitivity and function and energy surplus increase the supply of substrate to the liver, with fatty acids being esterified to glycerolipids and lipogenic substrates fuelling the DNL pathway. Adopted from Dearlove and Hodson (2022). Created with BioRender.com

2.4.8 Fructose, metabolic health and NAFLD

In addition to obesity and insulin resistance, fructose has also been associated with the development of NAFLD (Tappy, 2018; Yki-Järvinen et al., 2021). The consumption of fructose has increased over the last four decades and paralleled that of the coexisting epidemics of obesity, T2DM and NAFLD (Jin & Vos, 2015; Tappy & Lê, 2010). Unlike glucose, fructose is predominantly metabolised in the liver, and to a lesser extent in the small intestine and kidneys (Gonzalez & Betts, 2018; Tappy, 2018). The acute ingestion of fructose within a mixed meal (including carbohydrate, fat and protein) produces a less pronounced insulin response compared to glucose, whilst increasing postprandial lactate and TG concentrations (Chong et al., 2007). This is physiologically meaningful as the

lower insulin response following fructose ingestion may reduce TG clearance into adipose tissue (Chong et al., 2007; Sadur & Eckel, 1982) and promote hepatic DNL by providing non-lipid fuels (including both fructose and lactate) and upregulating transcriptional pathways (Hengist et al., 2019). It is therefore perhaps not surprising that fructose has been linked with poor metabolic health (Tappy, 2018). Moreover, a reduction in fructose intake, in addition to weight loss and exercise, are advocated for the management and treatment of NAFLD (Marchesini et al., 2016). The discussion to follow will provide an overview of dietary carbohydrate intake with a specific focus on fructose, its digestion, absorption, and metabolism, before discussing the acute effects of fructose consumption on postprandial metabolic responses and the role of exercise in attenuating these excursions.

2.4.9 Overview of dietary carbohydrate intake

Dietary guidelines recommend that carbohydrates should comprise approximately 50% of total daily energy intake (Buyken et al., 2018; SACN., 2015). All carbohydrates contain carbon, hydrogen and oxygen and are classified according to their chain length, constitute monomers and glycosidic bonds and include monosaccharides such as glucose, fructose and galactose; disaccharides including maltose (glucose-glucose), sucrose (glucose-fructose) and lactose (glucose-galactose); oligosaccharides (maltodextrin) and polysaccharides (starch) (Gonzalez et al., 2017; SACN, 2015). Monosaccharides and disaccharides are dietary sugars and can be further classified into ‘intrinsic sugars’ and ‘free sugars’ (WHO, 2015). The latter includes monosaccharides and disaccharides added to foods and beverages by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices and fruit concentrates, as defined by the WHO (2015) and subsequently adopted by the UK (SACN, 2015). However, there is no consensus as to the use of universal terms or definitions (Azaïs-Braesco et al., 2017; Buyken et al., 2018; SACN, 2015).

The most abundant dietary sugars are sucrose, lactose and their monomers (SACN, 2015; Stehle, 2014). For example, data from the UK estimate that sucrose accounts for approximately 50% of total sugar intake with a mean daily consumption of 40-50 g. Consumption of lactose, and glucose and fructose (excluding sucrose) was 10-13 g and 15-18 g per day, respectively (SACN, 2015). Mean total sugar intake accounts for 20% of total energy intake in older children and adults in the UK (SACN, 2015) and is comparable to other European nations (Azaïs-Braesco et al., 2017). This is despite recommendations by the WHO to reduce free sugar intake to less than 10% of total energy intake and advocating a further reduction to less than 5% of total energy intake (SACN, 2015; WHO, 2015). These recommendations are based on cross-sectional associations between free sugar intake, positive energy balance, obesity and the development of several chronic non-communicable disease states (SACN, 2015; WHO, 2015). Specifically, fructose has received increasing research attention in recent years and has been associated with the development of obesity, T2DM and NAFLD (Abdelmalek et al., 2010; Jensen et al., 2018; Tappy, 2018).

2.4.9.1 Fructose consumption

The consumption of sugar (sucrose) at a population level was low prior to the 18th century, before which it remained the reserve of the wealthy (Pereira et al., 2017; Tappy & Lê, 2010). However, the advent of intercontinental trade and advancing technologies to extract and refine sugar, initially from cane and later prepared from beets, resulted in sugar no longer being considered a luxurious commodity (Johnson et al., 2007; Tappy & Lê, 2010). Sugar was first added to tea and coffee as a sweetener before being used widely in the production of confectionary (Pereira et al., 2017; Tappy & Lê, 2010). Consequently, the average per capita sugar consumption in England increased from 1.8 kg to 8.1 kg between the years 1700 to 1800 and increased further to a mean intake of 45 kg per person in 1950 (Johnson et al., 2007). More contemporary data suggest a further exponential

increase to 100-150 kg per person per year in Europe, the United States and Australasia (Tappy & Lê, 2010; Tappy & Rosset, 2017).

Fructose continues to be consumed primarily in the form of sucrose in the UK and Europe (Pietinen et al., 2010; SACN, 2015; Stehle, 2014). In contrast, the introduction of high fructose corn syrup (HFCS) in the United States in the 1970s resulted in reduced sucrose consumption, concomitant to a rapid increase in HFCS intake (Tappy & Lê, 2010; Wells & Buzby, 2008). Unlike sucrose, HFCS is not composed of an equal amount of glucose and fructose. Instead, HFCS comprises a mixture of these monomers with 42% and 55% fructose, known as HFCS-42 and HFCS-55, respectively (Pereira et al., 2017; Tappy & Rosset, 2017). HFCS appealed to food manufacturers because of its stability in acidic foods and beverages, increased shelf life and lower cost (Johnson et al., 2007; White, 2008). Although sucrose remains the predominant source of added sugars in the United States (45%), HFCS contributes significantly to this total (41%) (Tappy & Lê, 2010).

In Europe, the main dietary sources of fructose include fruit, soft drinks, juices, cakes and confectionary (Roberts et al., 2018; Sluik et al., 2015). In patients with NAFLD, the consumption of fructose from soft drinks has been found to be two to three times higher than controls matched for age, sex, and BMI (Ouyang et al., 2008). Moreover, fructose consumption in the form of soft drinks has been associated with NAFLD in children, teenagers and adults, and correlates in a dose-dependent fashion with the severity of hepatic fibrosis (Abdelmalek et al., 2010; Jensen et al., 2018). The consumption of sugar sweetened beverages also increases the risk of NAFLD, with low (< 1 cup per week), moderate (1-6 cups per week) and high (≥ 7 cups per week) doses increasing the relative risk of NAFLD by 14%, 26% and 53%, respectively (Chen et al., 2019). Although association does not equate to causation, these data demonstrate consistent findings for the role of fructose consumption in the development of NAFLD.

2.4.9.2 Fructose digestion and absorption

Fructose is an isomer of glucose and is distinct from other hexoses because of the presence of a keto group in position 2 of its carbon chain (Tappy & Lê, 2010). All carbohydrates must first be hydrolysed into their respective monomers prior to transepithelial transport across the brush-border and basolateral membranes, respectively (Daniel & Zietek, 2015; Gonzalez et al., 2017). The digestion of carbohydrates is initiated in the oral cavity by the secretion of salivary enzymes, of which alpha-amylase is the most abundant (Bornhorst & Singh, 2012). Carbohydrate sensitive receptors in the oral cavity stimulate regions of the cerebrum associated with reward, motor drive and improved exercise performance (Chambers et al., 2009). The sensing of carbohydrate and other macronutrients in the mouth and the subsequent anticipation of incoming nutrients also causes up-regulation of downstream endocrine responses including insulin release; referred to as cephalic phase insulin release (Just et al., 2008; Teff, 2010). When ingested alone, carbohydrate digestion is rapid and presents substrates to the intestine for absorption (Gonzalez et al., 2017; Wallis & Wittekind, 2013). Furthermore, gastric emptying of a specific monosaccharide may be accentuated by acute feeding of that specific monomer (Yau et al., 2017).

Sucrose is hydrolysed to glucose and fructose in the intestine via saccharidases bound to the brush-border-membrane (Daniel & Zietek, 2015). Fructose is absorbed from the intestinal lumen to the cytosol of the enterocyte passively via the intestinal glucose transporter (GLUT) GLUT5 (Drozdowski & Thomson, 2006; Ferraris, 2001). Additional transporters may also facilitate fructose absorption, but these are not thought to contribute significantly to this process (DeBosch et al., 2012; Gonzalez et al., 2017). As a consequence, fructose absorption is finite, with fructose malabsorption occurring at low doses (Douard & Ferraris, 2008; Tappy & Lê, 2010). Fructose is subsequently transported from the enterocyte across the basolateral membrane to the portal circulation via GLUT2

(Drozdowski & Thomson, 2006; Ferraris, 2001), where it is metabolised primarily by the liver (Tappy & Lê, 2010).

In contrast, the transepithelial absorption of glucose and galactose is mediated via the apical sodium dependant glucose transporter 1 (SGLT1) and exit the enterocyte facilitated by GLUT2 (Drozdowski & Thomson, 2006; Ferraris, 2001). Once released into the systemic circulation glucose can be utilised by extra-splanchnic tissues. Galactose on the other hand must first enter the liver to be metabolised like that of fructose (Gonzalez et al., 2017).

2.4.9.3 Fructose metabolism

Fructose is metabolised primarily in the liver but also in other splanchnic organs including the proximal intestine and kidney tubules (Gonzalez & Betts, 2018; Tappy & Lê, 2010). Hepatic fructose metabolism, known as fructolysis, is well characterised and has been described in detail previously (Figure 2.6) (Mayes, 1993; Tappy & Lê, 2010; Tappy & Rosset, 2017). The liver is a unique and highly specialised metabolic organ that expresses all the enzymes necessary for the metabolism of substrates that cannot be used directly by the majority of cells, including amino acids, alcohol, galactose and fructose (Tappy & Rosset, 2017). Once presented to the liver in the portal vein, fructose is efficiently extracted by hepatocytes via GLUT2 and rapidly metabolised to fructose-1-phosophate catalysed by fructokinase (Mayes, 1993; Tappy & Lê, 2010). The isoform of fructose kinase localised to the liver (also known as ketohexokinase C [KHK-C]) is highly expressed in hepatocytes and has a high affinity for fructose (Diggle et al., 2009), hence its swift conversion. Fructose-1-phosophate is subsequently metabolised to triose-phosphates (glyceraldehyde and di-hydroxy-acetone-phosphate [DHAP]) under the action of aldolase B (Mayes, 1993; Tappy & Lê, 2010). Finally, triokinase converts glyceraldehyde into glyceraldehyde-3-phospahte [GAP] (Tappy & Lê, 2010; Tappy &

Rosset, 2017). The products of fructolysis (GAP and DHAP) are also intermediates of glycolysis and share subsequent metabolic steps (Tappy & Rosset, 2017). However, in contrast to glycolysis, in which the inhibitory effect of increased adenosine tri-phosphate (ATP) and citrate turnover regulate the activity of phosphofructokinase, fructolysis bypasses this rate-limiting step (Mayes, 1993; Tappy & Lê, 2010). As a consequence, the metabolism of triose-phosphates in fructolytic cells is largely unregulated and directly proportional to fructose uptake (Tappy, 2021; Tappy & Lê, 2010). Triose-phosphates may subsequently be converted into glucose, lactate and acetyl coenzyme A (Acetyl Co-A) (Tappy & Rosset, 2017), thereby providing precursors to hepatic DNL for the synthesis of new fatty acids (Hengist et al., 2019).

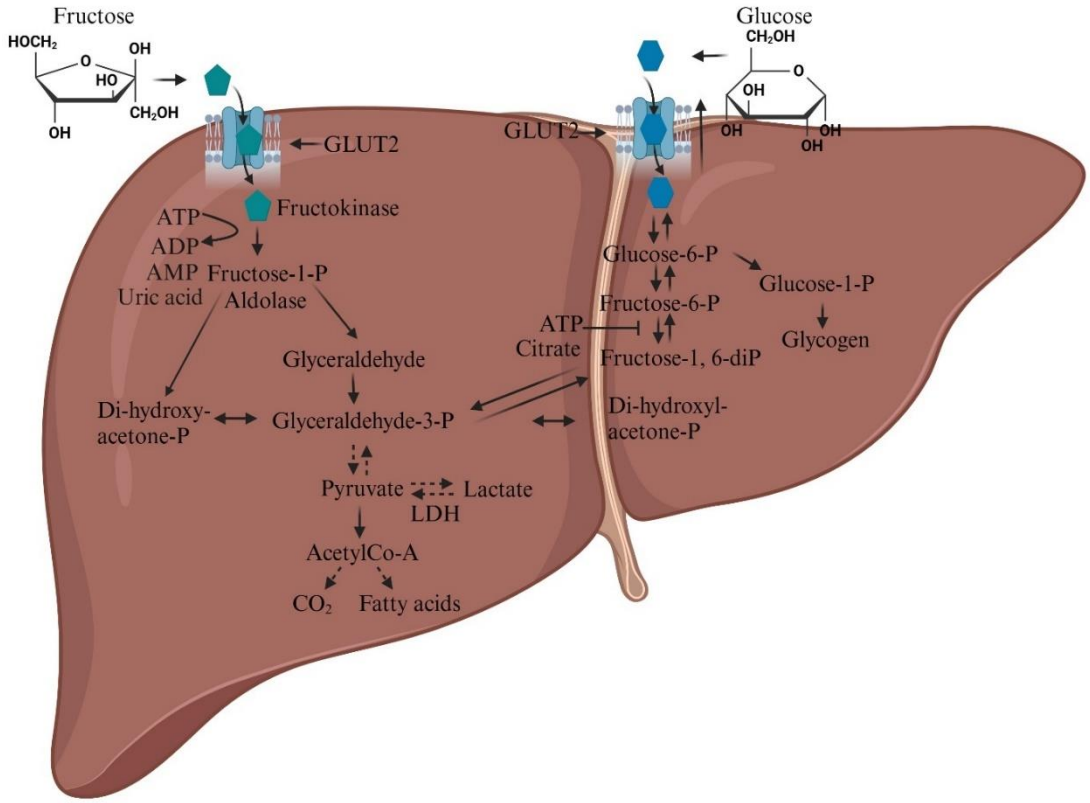

Figure 2.6 Differences in hepatic fructose and glucose metabolism. Acetyl Co-A, acetyl coenzyme-A; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CO_2, carbon dioxide; GLUT2, glucose transporter 2; LDH, lactate dehydronase; P, phosphate. Adapted from Tappy and Le (2010). Created with BioRender.com

Fructose may also directly stimulate hepatic DNL by the upregulation of transcription factors and genes needed for fatty acid and TG synthesis (Hengist et al., 2019; ter Horst & Serlie, 2017). Fructose can activate the transcription factors sterol regulatory element binding protein 1c (SREBP-1c) and carbohydrate-responsive element-binding protein (ChREBP) (Samuel & Shulman, 2016; ter Horst & Serlie, 2017). These factors may be further amplified by peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator 1β; a transcriptional coactivator for SREBP-1c as a result of fructose ingestion (Low et al., 2018; Nagai et al., 2009). In a well-designed study, ter Horst and associates (2021) demonstrated that fructose robustly stimulates hepatic DNL in NAFLD patients and obese controls. The expression of ChREBPβ, the potent isoform of ChREBP, was also increased in NAFLD patients and provides evidence for the direct effect of fructose feeding on ChREBP in human liver (ter Horst et al., 2021; ter Horst & Serlie, 2017). In addition, fructose-1, 6-bisphosphate, an intermediate in the fructolysis pathway, may combine with the triose-phosphate glyceraldehyde to synthesise xylulose-5-phosphate (Bonsignore et al., 1962), and subsequently activate protein phosphatate 2A (Kabashima et al., 2003), a further activator of ChREBP (Dentin et al., 2006; Low et al., 2018).

Although hepatic fructose metabolism is well described and thought to be the primary site of fructose handling, the intestine also possesses all the enzymes required for fructose metabolism (Steenson et al., 2017). Contemporary evidence in both rodents (Jang et al., 2018, 2020) and humans (Steenson et al., 2020) has demonstrated that enterocytes may contribute to fructose metabolism more so than previously thought. For example, Jang and colleagues (2018) reported that ~ 90% of fructose phosphorylation, the first step in the pentose pathway, takes place in the small intestine. By sampling blood from the hepatic vein, the group also showed that low-dose fructose ingestion (< 0.5 g·kg) resulted in the majority of fructose being presented to liver as glucose and lactate (~ 60%), whilst

less than 20% escaped intestinal metabolism (Gonzalez & Betts, 2018; Jang et al., 2018). Further increasing the fructose dose to ≥ 1.0 g·kg saturated intestinal fructose capacity and exposed the liver to higher fructose concentrations (Jang et al., 2018). In contrast, the human proximal intestine and liver are relatively small and may explain why fructose doses a low as ~ 0.1 g·kg and 0.33 g·kg may increase circulating fructose concentrations and stimulate DNL, respectively (Gonzalez & Betts, 2018; Moore, 2019; Tran et al., 2010). Therefore, at low doses, intestinal fructose metabolism may be protective and shield the liver directly from fructose (Jang et al., 2018, 2020). However, the appearance of glucose and lactate may still provide non-lipid fuels for hepatic DNL, thereby contributing to metabolic disease (Jang et al., 2018).

Recently, Steenson and co-workers (2020) published novel data that quantified the rate of intestinal DNL in humans for the first time. No differences in intestinal DNL were reported between low (~ 0.14 g·kg) and high (~ 1.77 g·kg) fructose feeding interventions; with the respective contributions to chylomicron-TGs only minor. In absolute terms, volunteers ingested ~ 13g / < 2% of energy (low) and ~ 165 g / 30% of energy (high) of fructose over an 11-hour period. These findings appear to add support to the observation that intestinal fructose metabolism is saturated at low doses in mammals (Gonzalez & Betts, 2018; Jang et al., 2018). Indeed, when scaled to body surface area, humans may saturate the small intestines capacity for fructose metabolism at ~ 5 g, approximately a quarter of a banana (Gonzalez & Betts, 2018). Although further investigations are needed, the contribution of the intestine to fructose metabolism at intakes more akin to habitual consumption may therefore be limited, shifting the emphasis back to the liver and alternative mechanisms (Gonzalez & Betts, 2018). Steenson and colleagues (2020) also measured hepatic DNL and similarly found no differences between the low and high fructose interventions or the contribution to VLDL-TGs.

However, high fructose feeding did result in significantly increased systemic TG concentrations, including plasma TGs, VLDL-TGs, and NEFAs; whilst insulin concentrations were lower (Steenson et al., 2020). This data, coupled with the previous observations of others, suggest that excess fructose consumption reduces fatty acid clearance, as opposed to increasing production (Chong et al., 2007; Steenson et al., 2020; Watkins et al., 2020). This appears to be mediated by the lower postprandial insulin response to fructose feeding, which may reduce TG clearance by a number of mechanisms, including lower LPL activation and TG uptake into adipose tissue (Chong et al., 2007; Sadur & Eckel, 1982); reduced suppression of adipose tissue lipolysis and continued supply of NEFAs to the liver (Frayn et al., 1993; Jensen et al., 1989); and reduced inhibition of hepatic VLDL-TG production (Lewis et al., 1995). The postprandial responses to acute fructose feeding are discussed in the following section.

2.4.10 Postprandial responses to acute fructose feeding

Poor metabolic health can be characterised by elevated fasting glucose and TG concentrations and represent two of the five criteria for the diagnosis of MetS as aforementioned (Alberti et al., 2009). Although fasting measurements have traditionally been used, contemporary guidance advocates the use of postprandial responses (Kolovou et al., 2019a; Nordestgaard et al., 2016). Following a mixed meal, plasma glucose and TG concentrations remained elevated for 2 hours and ~ 8 hours, respectively, in healthy individuals (Edinburgh et al., 2017). Assuming three to four eating occasions per day, most individuals spend the majority of their day in the postprandial state (Edinburgh et al., 2017). Postprandial measures therefore better reflect metabolic excursions in response to habitual eating patterns, with exacerbated responses associated with CVD, T2DM and NAFLD (Kolovou et al., 2011; Kolovou et al., 2019a).

Postprandial measurements are typically assessed in response to the OGTT or OFTT. In contrast to the established 75 g glucose load utilised in the OGTT (Alberti et al., 1985), different types, structure and amounts of fat, carbohydrate or protein have been included in the OFTT (Kolovou et al., 2011; Kolovou et al., 2019a). Some have attempted to standardise the OFTT, with an expert panel recommending 75 g of fat, 25 g of carbohydrates, and 10 g of protein. For example, 250 g of dairy cream with the addition of 15 g of table sugar (Kolovou et al., 2011). This guidance also highlights that macronutrients are in seldom consumed in isolation, with others advocating the use of mixed nutrient challenges (Stroeve et al., 2015). The addition of carbohydrate to an oral fat load augments postprandial lipaemia (Cohen & Schall, 1988; Grant et al., 1994; Saito et al., 2013). Moreover, the co-ingestion of fructose or fructose containing carbohydrates (i.e., sucrose) with fat further exacerbates the TG response (Cohen & Schall, 1988; Grant et al., 1994; Saito et al., 2013). This effect is perhaps not surprising given the postprandial responses to fructose when consumed alone (Jameel et al., 2014).

Cohen and Schall (1988) compared the postprandial metabolic responses to a meal containing 40 g of fat from dairy cream and the same meal with the addition of 50 g of glucose, fructose, or sucrose or 100 g of sucrose, respectively. Although others had previously investigated the effects of simple carbohydrates in addition to fat or a mixed meal on postprandial lipaemia (Albrink & Man, 1957; Mann et al., 1971; Nikkila & Pelkonen, 1966), the study by Cohen and Schall (1988) was the first to systematically compare these metabolic effects. Postprandial TG total area under the curve (tAUC) was significantly higher following consumption of the fat and fructose (50 g), and fat and higher sucrose (100 g) load. The authors suggested that the co-ingestion of fructose, including the fructose fraction within the higher sucrose meal, was responsible for the exacerbated TG tAUC because the postprandial response to both meals was similar whilst fat and glucose feeding attenuated the TG tAUC response (Cohen & Schall, 1988).

Unfortunately, glucose and insulin responses were only measured in a sub-group of participants during the latter study condition; therefore, it is challenging to draw further conclusions from a mechanistic perspective. Furthermore, the effects of the additional energy provided within the fat and higher sucrose meal are difficult to quantify in the absence of a calorie matched fat and glucose or fructose control; with the higher TG tAUC possibly an artefact of the additional energy content as opposed to fructose *per se*. However, when matched for carbohydrate and caloric content, the observations of Cohen and Schall (1988) demonstrate the lipogenic effects of fructose feeding compared to other simple carbohydrates.

Later, Singleton and colleagues (1999) similarly used an approximate 40 g oral fat load from diary cream to that of Cohen and Schall (1988), however with a fructose fraction more akin to habitual intake (30 g), as opposed to the 50 g fructose loads utilised previously (Cohen & Schall, 1988; Jeppesen et al., 1995). The magnitude of the TG tAUC increase following oral fat and fructose ingestion (with fat alone as the reference) was 38% compared to 75% reported by Cohen and Schall (1988). These data appear to demonstrate a dose-response relationship with higher TG tAUC responses with correspondingly higher fructose loads.

However, as aforementioned, it is challenging to delineate the metabolic effects of fructose alone when consumed in caloric surplus or not matched to a glucose or sucrose control. To this end, Chong and associates (2007) sought to determine the mechanisms for the acute effects of fructose on postprandial lipaemia by comparing energy matched fat and fructose, and fat and glucose solutions. Participants ingested 0.75 g·kg of either fructose or glucose in addition to 0.5 g·kg of oil (85% palm oil and 15% sunflower oil). Lower glycaemic and insulinaemic responses to fat and fructose ingestion were observed, in addition to higher postprandial TG excursions when compared to fat and glucose feeding. The authors concluded that the lower insulin response to fructose reduced

adipose tissue LPL activity and subsequently reduced plasma TG removal into adipocytes. Similarly, although the contribution of DNL to TG excursions following fructose feeding was small, this effect may contribute significantly to shifting the partitioning of fatty acids away from oxidation and towards esterification (Chong et al., 2007).

Saito and coauthors (2015) similarly employed a feeding strategy relative to body mass, however, in contrast to Chong et al. (2007) and in keeping with previous studies, utilised dairy cream as their fat source. Volunteers consumed 0.50 g·kg of fructose and/or glucose combined with 1.0 g·kg dairy cream (0.35 g·kg of fat). Fructose and glucose were provided as 100% fructose or glucose, or combined in the following fractions: 90% fructose and 10% glucose or 55% fructose and 45% glucose (Saito et al., 2015). This equated to approximately 26.5 g of fructose, glucose or fructose and glucose combined and 18.6 g of fat in absolute terms, respectively. The amount of fructose, glucose and fat supplied was therefore significantly less to that of earlier work (Cohen & Schall, 1988; Jeppesen et al., 1995). Despite these differences, the authors observed pronounced TG incremental AUC (iAUC) responses to the fat and higher fructose (100% fructose and 90% fructose, 10% glucose mixture) compared to combined fat and glucose solutions. These observations were again accompanied by lower insulin excursions. Moreover, the iAUC for hepatic TG-rich lipoproteins and chylomicrons remnants, indicative of endogenous and exogenous lipaemia, were increased post fat and high fructose ingestion compared to fat and glucose feeding (Saito et al., 2015). The study of Saito and colleagues therefore adds further support to the proposed mechanisms of fructose induced lipaemia as aforementioned, i.e., reduced TG clearance into adipocytes and partitioning of fatty acids towards esterification within the liver (Chong et al., 2007).

More recently, Gallagher and associates (2016) compared the postprandial responses to fat and fructose, and fat and sucrose in the form of a solid meal in a heterogeneous cohort of apparently healthy, overweight, and obese volunteers. This is noteworthy because

previous studies, with the exception of the early work of Cohen and Schall (1988), have compared fructose and glucose whereas habitually these monosaccharides are primarily consumed as sucrose in the form of confectionary and sugar sweetened beverages (Roberts et al., 2018; SACN, 2015). Therefore, comparing fructose and sucrose in solid form strengthens the ecological validity of the study. Fructose (52 g) and sucrose (65 g) were matched for sweetness, as opposed to energy content, and were delivered in the form of muffins with a total fat content of 66 g. In contrast to previous studies, the TG tAUC or iAUC responses were not significantly different following fructose or sucrose feeding. However, glycaemic and insulinaemic responses were lower after fructose compared to sucrose ingestion. As discussed by the authors, a caveat of the study was the additional energy content within the sucrose meal. Although the difference was small (27 k·cal in total), this included an increase in simple carbohydrates (13 g sucrose) whilst reducing the starch content (3 g). Indeed, the overall carbohydrate content of the sucrose meal was 119 g (54 g starch) and the fructose meal 109 g (57 g starch). It is therefore difficult to draw conclusions as to the underlying mechanisms given the disparities in both energy and carbohydrate content, and in the absence of other metabolic parameters. The physical structure of the meal is also an important consideration. It would be interesting if the authors were to extend their work to compare energy and carbohydrate matched solid and liquid meals and the subsequent effect on postprandial metabolic responses.

In summary, the addition of simple carbohydrates to an oral fat solution further augment the postprandial TG response compared to fat alone (Cohen & Schall, 1988; Grant et al., 1994; Saito et al., 2013). However, observations concerning the comparison of glucose, fructose, and sucrose in addition to a fat load are equivocal. This is largely due to the disparities in meal composition, including the total amount of fat, carbohydrate and energy provided; whether these are matched for macronutrient or caloric content or both; the use of absolute or relative feeding strategies; and the physical structure of the meal,

i.e., solid versus liquid. Overall, there is evidence that the addition of fructose or fructose containing carbohydrates to an oral fat load exacerbates postprandial lipaemia compared to glucose. The underlying mechanism appears to be the reduced insulin response to fructose ingestion and the diminished clearance of plasma TGs (Chong et al., 2007).

2.4.11 Exercise as a strategy to attenuate postprandial responses to acute fructose feeding

It is well established that prior exercise can reduce postprandial metabolic excursions to a subsequent meal (Burns et al., 2015; Freese et al., 2014; Pearson et al., 2022). Exercise performed 8-24 hours before a high-fat meal reduces the magnitude of the TG response by 20%-25% (Burns et al., 2015; Freese et al., 2014; Pearson et al., 2022). Experimental evidence suggests that exercise increases LPL activity and subsequent TG clearance and reduces hepatically synthesised TG-rich VLDL (Gill et al., 2001; Herd et al., 2001; Rabøl et al., 2011). However, despite these beneficial effects, few studies have examined the potential of acute exercise to attenuate the TG response to concurrent fat and fructose ingestion (Macedo et al., 2019; Rowe et al., 2016; Wilburn et al., 2015).

For example, Macedo and colleagues (2019) reported a ~ 30% reduction in the TG tAUC to a fat and fructose meal following aerobic exercise compared to rest. The meal was designed to mimic that of a fast-food meal with a sugar sweetened beverage. The energy content of the meal was calculated as 50% of each participants resting metabolic rate (RMR) as measured via indirect calorimetry and provided approximately 48 g of fat; whilst fructose was calculated relative to body mass (0.50 g·kg, mean intake of 34.6 ± 5.4 g). Participants walked for 45 minutes at 60% of peak oxygen uptake ($\dot{V}O_{2peak}$) approximately 13 hours prior to ingestion of the test meal. The reduction in TG tAUC was accompanied by improved insulin sensitivity (homeostatic model of insulin resistance [HOMA-IR]) following acute prior exercise. Mechanistically, this may have

improved TG uptake by adipocytes and skeletal muscle whilst suppressing hepatic VLDL output.

Conversely, Rowe and associates (2016) documented no improvements in TG excursions following acute exercise in response to mixed meals containing fructose or when compared to the same meal in which the fructose fraction was exchanged with glucose. Akin to the previous study of Macedo et al. (2019), the research group employed a similar exercise protocol in which participants walked at 70% $\dot{V}O_{2max}$ approximately 14-16 hours the evening prior to the test meal, before returning to the laboratory the following morning. However, rather quantifying exercise by time, volunteers terminated exercise once they had expended 500 k·cal. Further disparities included the fat and carbohydrate content of the test meals (approximately 27 g of fat and 61 g of fructose or glucose, respectively) which were calculated according to fat-free mass rather than as a percentage of RMR. Insulin tAUC was however significantly reduced to both fructose and glucose containing meals following acute exercise, suggesting an improvement in insulin sensitivity (Rowe et al., 2016). The authors concluded that a greater amount of energy may have needed to be expended to further reduce the TG response (Rowe et al., 2016). Indeed, the attenuation of postprandial TG excursions appears to be largely dependent on exercise energy expenditure, with a value between 1.5-2.5 megajoule (MJ) conferring beneficial effects (Pearson et al., 2022).

A further consideration is the replacement of energy post-exercise. In the above cited studies participants consumed a standardised meal under supervision (Macedo et al., 2019) or were simply asked to replicate their eating behaviour prior to each trial (Rowe et al., 2016). Therefore, it may not be energy expenditure *per se*, but rather the creation and maintenance of an energy deficit that influences the efficacy of exercise interventions to reduce postprandial TG concentrations (Freese et al., 2011; Miyashita et al., 2020). To this end, Wilburn and colleagues (2015) investigated the effects of acute resistance

exercise with or without energy replenishment on postprandial responses to a fat (~ 39 g) and fructose (~ 59 g) solution. Postprandial TG excursions were significantly reduced following exercise compared to the non-exercise control condition (~ 20%); however, there were no significant differences between exercise with and without energy replenishment. Although not statistically significant, TG concentrations were reduced by approximately a third without (24.4%) compared to with (16.5%) post-exercise energy compensation (Wilburn et al., 2015).

Collectively, these data demonstrate the myriad of methodological disparities between existing studies and provide important considerations for those planning future investigations examining the effects of acute exercise on postprandial responses to concomitant fat and fructose ingestion. Of note, the highlighted studies have typically recruited apparently healthy individuals. It would be interesting to replicate or extend this work in those who are inactive, overweight or obese given the health effects of fructose appear to be linked to physical inactivity and low energy turnover (Tappy & Rosset, 2019; Walhin et al., 2021). Individuals with multiple components of MetS, and therefore at greater risk of NAFLD, of which exercise is the cornerstone of treatment, may be of particular benefit from the outcomes of such research.

2.5 Summary and study aims

The liver may be considered the chief metabolic organ. It coordinates the mobilisation, synthesis and storage of monosaccharides and lipids in the transition from the fasted to fed state and *vice versa*. Disturbances in these pathways can cause the accumulation of IHTG from both lipid and non-lipid sources, which is the defining feature of NAFLD. The emergence of NAFLD has paralleled that of obesity and T2DM, with an estimated global prevalence of 30%. Despite this, NAFLD is under-recognised in primary care and is often an incidental finding. Several predictive algorithms consisting of simple

anthropometric and biochemical/haematological parameters have therefore been developed to identify those at risk of NAFLD and hepatic fibrosis. These predictive panels may also be used in research settings for the population-based study of NAFLD. However, the use of such panels for this purpose is limited, particularly in unselected cohort studies. Chapter 4 therefore sought to determine the prevalence of NAFLD and the presence/risk of hepatic fibrosis/advanced fibrosis using several established composite panels reviewed above (Composite panels for identifying steatosis and Composite panels for identifying and staging fibrosis). This chapter also examined the association between predictors of metabolic risk, hepatic steatosis and fibrosis within the same cohort. Lifestyle changes, including increased physical activity and dietary modification are advocated for the treatment and management of NAFLD. Regarding the latter, excess fructose consumption has been associated with poor metabolic health and acutely increases postprandial TG concentrations, an established risk factor cardiometabolic disease, including NAFLD. Moreover, the addition of fructose to a high fat meal, such as a metabolic challenge, further exacerbates TG excursions. Exercise is an increasingly evaluated method to attenuate these undesirable postprandial TG perturbations. However, data pertaining to the efficacy of acute exercise to ameliorate TG and glucose responses, in both healthy individuals and those living with overweight/obesity and displaying several components of MetS, and therefore at risk of NAFLD, is relatively limited. To address this, Chapters 5 and 6 examined the inclusion of fructose in an OFTT, in the form of fructose only or as sucrose (glucose-fructose). This was preceded by rest or prior evening exercise to determine the efficacy of this intervention in attenuating postprandial metabolic responses in separate cohorts of healthy (Chapter 5) and inactive, overweight adult males (Chapter 6).

Chapter 3 General Methods

This chapter will outline the general methods common to the two experimental studies included in this thesis (Chapter 5 and Chapter 6, respectively).

3.1 Ethical approval

All experimental procedures were prior approved by the Faculty of Health Sciences Research Ethics Committee (FHS REC), the University of Hull (REF FHS83, Appendix 1, and REF FHS161, Appendix 2, respectively) and in accordance with the Declaration of Helsinki. Participants completed a pre-exercise medical questionnaire and provided their written informed consent before any experimental procedures were undertaken. For the second intervention study (Chapter 5), participants received an incentive of a £50 Amazon voucher or a £50 donation to a charity of their choice on completion of all study visits. The funding was provided by Mr Richard Page.

3.2 Study design

Both experimental studies followed a randomised, single blind, repeated measures research design and comprised of one screening and four intervention visits. Randomisation occurred *a priori* using a pseudo-random number generator (https://www.randomizer.org/). Participants received no information regarding the type of carbohydrate (i.e. sucrose or fructose) included in the non-proprietary meals at interventional visits.

3.3 Participant recruitment

Participants were recruited through word of mouth, poster and email advertisements from the University of Hull staff and student population. All poster and email adverts received prior approval from the FHS REC. Posters were placed on notice boards across the

University estate. Email advertisements were communicated via the staff 'ebulletin', the FHS staff email list and the postgraduate student email list. Posters and email adverts for Chapter 6 can be found in Appendix 3 and Appendix 4, respectively.

All experimental procedures were undertaken in the Applied Physiology Laboratories, Washburn Building, the University of Hull. Specific information pertaining to each experimental study, including the inclusion and exclusion criteria; participant characteristics; periods of recruitment and data collection; sample size estimation; and statistical analysis are detailed in Chapter 5 and Chapter 6, respectively.

3.4 Interventions

Participants visited the laboratory on five occasions in total (Figure 3.1). This included an initial screening visit and four subsequent visits to undertake each of the four study interventions: (1) an abbreviated OFTT with the addition of sucrose following rest (OFTT-Sucr); (2) OFTT with the addition of sucrose following prior evening exercise (OFTT-Sucr-Ex); (3) OFTT with the addition of fructose following rest (OFTT-Fruc); and (4) OFTT with the addition of fructose following prior evening exercise (OFTT-Fruc-Ex). The nutritional composition of the mixed nutrient metabolic challenges are described below (see Mixed nutrient metabolic challenge). On two occasions (OFTT-Sucr-Ex and OFTT-Fruc-Ex), participants completed a prior evening exercise session which commenced between 16:00 and 19:00, before returning to the laboratory the following morning to undertake the remaining experimental procedures as part of the visit. Nutritional intake the evening prior to the mixed nutrient challenges was standardised by providing participants with a convenience meal that was consumed before 20:00 (see Prior evening meal). All other laboratory visits were scheduled prior to 09:30 which participants attended following an overnight 12 hour fast. Participants were instructed to refrain from strenuous physical activity/exercise and alcohol 24 hours prior to each visit.

All laboratory visits were separated by at least 72 hours and completed within eight weeks of the screening visit.

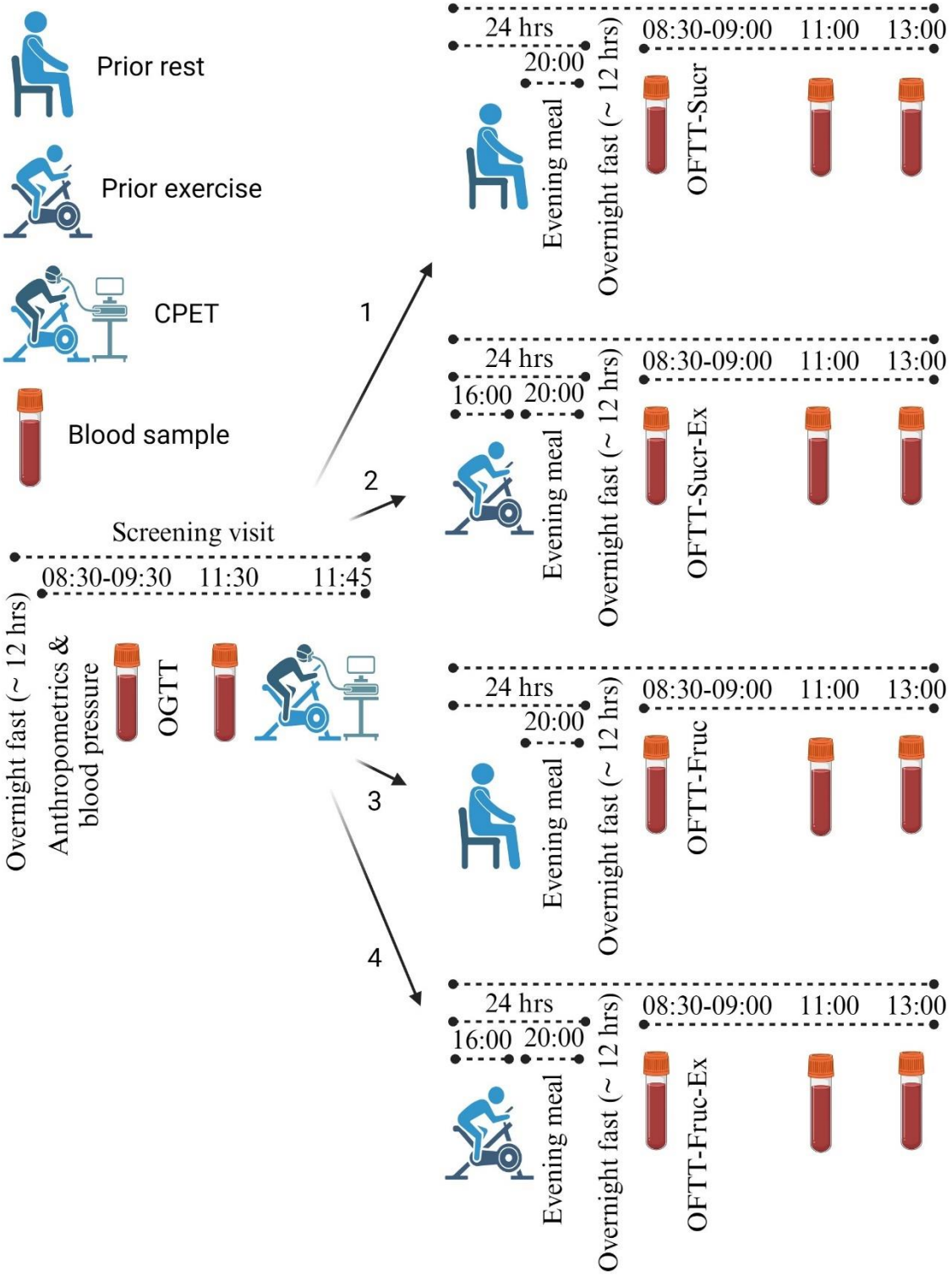

Figure 3.1 Schematic representation of the study protocol for Chapters 5 and 6. CPET, cardiopulmonary exercise test; OFTT-Sucr, oral fat tolerance test with sucrose; OFTT-Sucr-Ex, oral fat tolerance test with sucrose OGTT and prior exercise, OFTT-Fruc, oral fat tolerance test with fructose; OFTT-Fruc-Ex, oral fat tolerance test with fructose and prior exercise; OGTT, oral glucose tolerance test; hrs, hours. Created with BioRender.com

3.5 Screening visit

Briefly, the screening visit consisted of the determination of baseline anthropometric characteristics; blood pressure; an OGTT; and a cardiopulmonary exercise test (CPET).

3.5.1 Anthropometry

Body mass was determined to within 0.1 kg (SECA balance scales, Vogel & Halke, Hamburg, Germany) with participants wearing sports attire following the removal of footwear and personal items. A measurement was recorded when the balance was stationary and adjacent to the beam. Height was recorded to the nearest mm using a wall-mounted Stadiometer (Holtain Limited, Crymych Dyfed, Wales, UK). Participants were instructed to remove footwear and stand upright with their heels, buttocks, upper back and head in contact with the stadiometer. The head was oriented to the correct anatomical position by the test administrator (Frankfort plane). Height was recorded following a deep inhalation. BMI was calculated using the following formula:

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

Waist and hip circumferences were obtained using a flexible, non-elastic tape (SECA 201 measuring tape, Vogel & Halke, Hamburg, Germany). Both measurements were recorded with the participant standing with feet together, arms by the side of the body and abdomen relaxed. Waist circumference measurements were acquired by placing the tape immediately above the right iliac crest and taking a measurement in the horizontal plane with the tape in contact with the skin (Pescatello et al., 2014). This anatomical landmark was selected because of the ease of which it could be identified by palpitation so that the measurement could be standardised (Pi-Sunyer et al., 1998). Hip circumference was measured at the widest protuberance of the buttocks (Pescatello et al., 2014). Measurements were performed in duplicate and the mean reported. The waist to hip ratio

(WHR) (1) and waist to height ratio (WHtR) (2) were calculated using the following formula, respectively:

$$\text{WHR} = \text{waist circumference (cm)} / \text{hip circumference (cm)}$$

$$\text{WHtR} = \text{waist circumference (cm)} / \text{height (cm)}$$

Body fat percentage was estimated using bio-electrical impedance (BF900 Maltron Body Composition Analyser, Maltron International Limited, Rayliegh, Essex, UK). Participants were orientated in the supine position with legs spaced slightly apart and the right arm positioned approximately 10 cm away from the body. Electrodes (HAB Limited, UK) were positioned in the standard tetrapolar arrangement. All electrodes were placed on the right side of the body. Prior to placement, excess body hair was removed if necessary and the site cleansed (Alcotip pre-injection swab, Bunzl Retail and Healthcare Supplies Limited, UK). One electrode was placed on the wrist between the medial and lateral styloid process and a second distally on the hand immediately above the metacarpal-phalangeal joint in the second space between the metacarpals. Further electrodes were placed on the ankle between the medial and lateral malleoli and another distally on the foot just above the metatarso-phalangeal joint in the second metatarsal space. Participant data, including sex, height, mass, age, ethnic origin and activity level (‘normal’, ‘sport’ or ‘athletic’) were inputted, and a measurement acquired. A duplicate measurement was acquired immediately with the electrodes remaining in-situ and participant data again inputted. The mean of the two measurements was reported.

3.5.2 Blood pressure measurement

A resting blood pressure measurement was recorded using an automated blood pressure monitor (Omron M6 digital automatic blood pressure monitor, Omron Healthcare Europe, Hoofddorp, Netherlands). The blood pressure cuff was placed on the left upper arm and

aligned with the brachial artery. Participants remained seated throughout the procedure with the left arm resting on the bench top and blood pressure cuff at the approximate level of the heart. The first of two blood pressure measurements was recorded after five minutes of quiet rest and a subsequent measurement recorded after a further three minutes (Pescatello et al., 2014).

3.5.3 Blood sampling and storage

Blood samples were acquired aseptically from an antecubital forearm vein via venepuncture. Approximately 20 ml of whole blood was drawn at each sampling point. Ethylenediaminetetraacetic acid (EDTA) and sodium fluoride/potassium oxalate collection tubes (Vacuette Tube, Greiner Bio-One GmBH, Kremsmunster, Austria) were centrifuged (Heraeus Labofuge 400 R centrifuge, Thermo Scientific, UK) immediately at 2383 x *g* for 15 minutes at 4 degrees Celsius (°C). Serum separating tubes (SSTII, Vacuette Tube, Greiner Bio-One GmBH, Kremsmunster, Austria) were allowed to coagulate at room temperature for 30 minutes before being centrifuged at 1992 x *g* for ten minutes at 4°C. The supernatant was aliquoted into cryovials and stored at -80°C awaiting further analyses (see Biochemical analysis).

3.5.4 OGTT

A fasting venous blood sample was acquired from a forearm vein prior to oral ingestion of a 75 g dextrose load (Bulk Powders, Colchester, UK) weighed using digital bench top scales (Kern EW 120-4NM electronic bench top scales, Kern and Sohn, GmBH, Belingen, Germany) and diluted in 300 ml of cold water. Participants were instructed to consume the solution within five minutes and rested quietly prior to a second venous blood sample that was drawn 120 minutes post-ingestion (Alberti et al., 1985). Plasma glucose was determined immediately prior to and 120 minutes post OGTT using a point-of-care analyser (see Biochemical analysis) for the fulfilment of study specific

inclusion/exclusion criteria (Chapter 5 and Chapter 6, respectively). Impaired fasting glucose (fasting plasma glucose [FPG] value of 5.6-6.9 $mmol \cdot l^{-1}$) or impaired glucose tolerance (120 minute post OGTT plasma glucose value of 7.8-11.0 $mmol \cdot l^{-1}$) were considered indicative of prediabetes (America Diabetes Association, 2014). A FPG value $\geq$ 7.0 $mmol \cdot l^{-1}$ or plasma glucose $\geq$ 11.1 $mmol \cdot l^{-1}$ 120 minutes post OGTT was considered indicative of T2DM (American Diabetes Association, 2014).

3.5.5 CPET

Participants performed a continuous incremental ramp protocol to volitional exhaustion on an electromagnetically braked cycle ergometer (General Electronics Ergometer eBike, General Electronics Medical Systems, Milwaukee, USA) in accordance with established guidelines (Balady et al., 2010). Expired gas analysis was recorded breath-by-breath throughout the protocol. Peak oxygen uptake ($\dot{V}O_{2peak}$ [$ml \cdot kg^{-1} \cdot min^{-1}$]) was defined as the highest oxygen consumption ($\dot{V}O_2$) averaged over 30 seconds during the CPET (Midgley et al., 2007). Participants were deemed to have provided a maximal effort if two of the four following criteria were attained: a plateau in absolute $\dot{V}O_2$ (< 150 ml increase per minute) with increasing workload; achieving a heart rate (HR) of > 85% of age-predicted maximum heart rate; a respiratory exchange ratio (RER) of > 1.10 at volitional exhaustion and/or a rating of perceived exertion (RPE) > 17 on the 6-20 Borg scale (Taylor et al., 2015). The ventilatory anaerobic threshold (AT [$ml \cdot kg^{-1} \cdot min^{-1}$]) was identified using the modified V-slope method (Beaver et al., 1986) and confirmed using ventilatory equivalents (Whipp et al., 1986).

3.5.6 Online metabolic cart calibration

Expired gas analysis data were collected breath-by-breath using an online metabolic cart (Oxycon Pro, Jaeger, Germany) which was calibrated immediately prior to data acquisition. Calibration of ambient temperature (°C) and barometric pressure (millibar

[mbar]) were conducted by computer automated software (JLab, Lab Manager, V5.30.0). Relative humidity (%) was entered manually by the test administrator and measured using a digital barometer (ClimeMET, model CM9088, UK). The flow/volume sensor was calibrated using a 3 L syringe (Hans Rudolph 5530, USA) and offset values automatically calculated for accurate measurement of ventilator volumes. A two-point calibration of the oxygen (O_2) analyser (electro-chemical cell) and carbon dioxide (CO_2) analyser (thermal conductivity) using gases of known concentrations (O_2: 16.36%, CO_2: 4.49%, CryoService Limited, Worcester, UK) was performed to allow for accurate determination of oxygen utilisation and carbon dioxide production. Participant characteristics and anthropometric data were entered into the software database prior to CPET and assigned a unique study identification.

3.5.7 Participant preparation

Each participant received verbal instruction regarding the CPET protocol, including: work rate increments, an explanation of the RPE scale (Borg, 1998) and test termination criteria. Participants were also given the opportunity to ask any questions. HR (Polar T31 heart rate monitor, Polar Electro OY, Kempele, Finland) (beats per minute [$b \cdot min^{-1}$]) and RPE were recorded at alternate minutes during CPET until test termination. An oronasal mask (V2 mask, Hans Rudolph, USA) was then positioned to cover the participant's mouth and nose and secured in place using a harness (Headgear, Hans Rudolph, USA) to allow for the collection of minute ventilation ($\dot{V}_e$) and respiratory gas exchange data. The cycle ergometer was configured to the participant's preference to ensure that they were comfortable prior to commencing the exercise protocol.

3.5.8 Exercise protocol

The exercise protocol consisted of three minutes of rest whilst seated on the cycle ergometer and three minutes of unloaded cycling at a self-selected cadence; followed by

a continuous incremental ramp protocol to volitional exhaustion. Work rate increments were individualised to each participant at the discretion of the test administrator based on the sex, body mass, self-reported physical activity and familiarity of the participant with cycling; with the objective to achieve exhaustion within 8-12 minutes. Ramp increments ranged between 20 and 30 Watts per minute ($W \cdot min^{-1}$). Participants were advised to maintain a comfortable cadence above 60 rotations per minute ($r \cdot min^{-1}$) during the incremental work rate phase of the test. The CPET was terminated when participants were no longer able to maintain a cadence of 60 $r \cdot min^{-1}$ despite strong verbal encouragement from the test administrator. This was followed by a five-minute recovery phase of unloaded cycling at a self-selected cadence.

3.6 Interventional study visits

Participants attended the laboratory in the morning before 09:30 in a fasted state on four occasions to consume the mixed nutrient metabolic challenges. Participants confirmed that their health status had not changed following their previous visit and re-signed their original medical questionnaire to this effect. A fasted venous blood sample was acquired prior to consumption of the OFTT-Sucr or OFTT-Fruc meal. Subsequent blood samples were acquired two and four hours postprandially. Participants rested quietly in the laboratory in the intervening period between data collection points and were allowed to consume water *ad-libitum*.

3.6.1 High intensity interval exercise

A submaximal HIIE session consisting of alternating intervals in the moderate and severe intensity domains was performed on an electromagnetically cycle ergometer (Ergoline, LoveMecial, UK) the evening prior (16:00-19:00) to two of the experimental study visits in a randomised fashion (Figure 3.2). The moderate intensity domain encompasses work rates below oxygen consumption at the AT (Gaesser & Poole, 1996; Poole & Jones, 2012).

The severe intensity domain represents work rates that can be sustained for a finite period in which an oxygen consumption steady state is not attainable (Gaesser & Poole, 1996; Poole & Jones, 2012). Power output corresponding to the oxygen consumption at AT and the severe intensity domain were calculated from each individual's CPET. The exercise session consisted of five minutes of cycling at 20 W (warm-up) proceeded by alternating one minute intervals of moderate and severe intensity exercise totalling 40 minutes, followed by five minutes of cycling at 20 W (cool-down); similar to that previously utilised by our laboratory (O'Doherty et al., 2017) and depicted in Figure 3.2. Participants were encouraged to maintain a comfortable self-selected cadence throughout. HR (Ergoline BM-CS5EU, Germany) and RPE were recorded at minute five of the warm-up and cool-down and at alternate minute intervals during interval exercise, respectively. Exercise in the moderate intensity domain was performed at a power output corresponding to 80% of the AT. This was calculated by identifying the work rate at AT, subtracting two thirds of the ramp rate from CPET to account for the discrepancy between muscle bioenergetics and pulmonary gas exchange and calculating 80% of that value (O'Doherty et al., 2018; Whipp et al., 1986). The power output corresponding to the severe intensity exercise was calculated as 50% of the difference (Δ 50%) between the work rates at AT and $\dot{V}O_{2peak}$ and again subtracting two thirds of the ramp rate from CPET from that value.

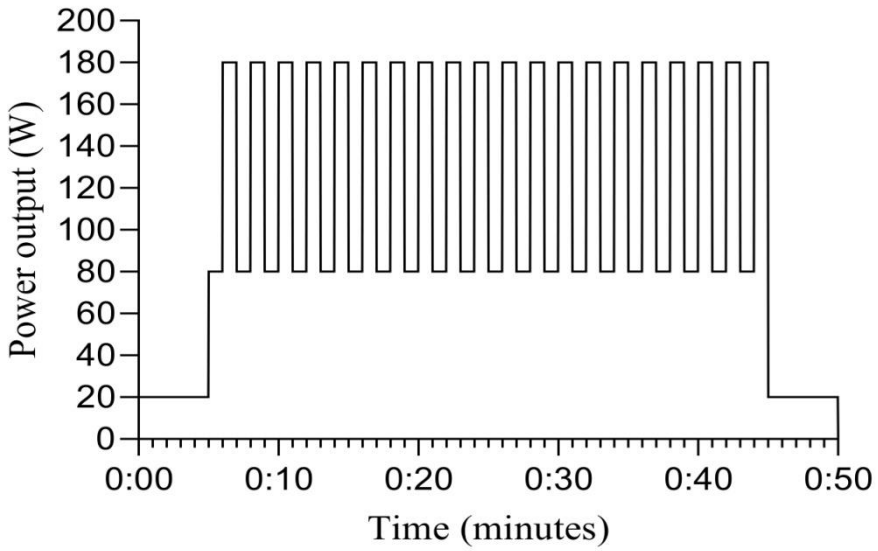

Figure 3.2 Graphical representation of the HIIE protocol.

3.6.2 Prior evening meal

Participants were asked to choose from one of three commercially available convenience meals (chicken tikka masala, spaghetti carbonara or macaroni and cheese, Tesco Stores Limited, UK) and consumed the same meal the evening prior to each visit following the screening visit. The energy and macronutrient content of each meal and the average (mean and SD) energy and macronutrient consumption of the convenience meals for Chapters 5 and 6 are shown in Table 3.1. For Chapter 5, seven participants selected the chicken tikka masala and one the macaroni and cheese. For Chapter 6, four of five volunteers choose the chicken tikka masala and one the macaroni and cheese. The meal was consumed prior to 20:00 to allow for a postprandial period of ≥ 12 hours when returning to the laboratory the following morning. Participants were instructed to consume no other nutrients in additional to the convenience meal that evening. This dietary control was necessary as the nutritional composition of evening meals may influence subsequent post challenge metabolic responses and is therefore an important consideration in the design of postprandial studies (Robertson et al., 2002).

Table 3.1 Energy and macronutrient composition of prior evening meals and average (mean) energy and macronutrient content of meals selected for Chapters 5 and 6, respectively.

	Chicken tikka masala	Macaroni and cheese	Chapter 5	Chapter 6
Energy (Kcal)	539	643	552 ± 37	559 ± 47
Fat (g)	17.0	22.1	17.6 ± 1.8	18.0 ± 2.3
Saturates (g)	5.8	11.9	6.6 ± 2.2	7.0 ± 2.7
Carbohydrates (g)	63.3	81.4	65.6 ± 6.4	66.9 ± 8.1
Protein (g)	29.3	27	29.0 ± 0.8	28.8 ± 1.0
Fibre (g)	7.7	5.1	7.4 ± 0.1	7.2 ± 1.2

Data are presented as mean and standard deviation (±). g, grams; Kcal, kilocalories.

3.6.3 Mixed nutrient metabolic challenge

The OFTT meals with the addition of either sucrose or fructose were designed specifically for this investigation and reflect the previous work of our laboratory (O'Doherty et al., 2018, Table 3.2). Mixed nutrient metabolic challenges were comprised of double cream (117.5 g, Tesco Stores Limited, UK), whole milk (257.5 g, Tesco Stores Limited, UK), chocolate drinking powder (20 g, Cadbury hot chocolate original, Mondelez, Birmingham, UK) and the addition of sucrose (60 g, granulated sugar, The Silver Spoon Company, Peterborough, UK) or fructose (60 g, Bulk Powders, Colchester, UK), respectively. All ingredients were weighed using digital bench top scales (Kern EW 120-4NM electronic bench top scales, Kern and Sohn, GmBH, Belingen, Germany) and served in a clear mixing container. Participants were required to ingest the liquid meal within 15 minutes and subsequently consumed an additional 100 ml of cold water added to the container to ensure that the meal was consumed in its entirety.

Table 3.2 Composition of the mixed nutrient metabolic challenges.

	OFTT-Sucr*	OFTT-Fruc†
Volume (ml)	375	375
Energy (Kcal)	1054	1054
Fat (g)	75	75
Saturates (g)	47	47
Carbohydrates (g)	81	81
Sucrose (g)	60	0
Fructose (g)	0	60
Protein (g)	14	14
Fibre (g)	2.2	2.2

*OFTT-Sucr: Oral fat tolerance test with the addition of sucrose; †Oral fat tolerance test with the addition of fructose. g, grams; Kcal, Kilocalories; ml, millilitres.

3.7 Biochemical analysis

3.7.1 Immediate analysis

Plasma glucose was determined immediately following centrifugation using a point-of-care analyser (Reflotron Plus, Roche Diagnostics Limited, Rotkreuz, Switzerland) to determine participant eligibility as part of the OGTT. Prior calibration and application of plasma (30 μl) to the magnetic test strips were performed in accordance with the manufacturer's guidelines.

3.7.2 Stored serum and plasma samples

An ABX Pentra 400 biochemistry autoanalyser (Horiba, Montpellier, France) was used for the determination of serum TG, total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), Apo B (Chapter 5 only), plasma glucose, alanine aminotransferase (ALT), aspartame aminotransferase (AST) and GGT. The bench top autoanalyser was calibrated and quality controls performed prior to use in accordance with the manufacturer's instructions and all samples were analysed in duplicate. Low density

lipoprotein cholesterol (LDL-c) was estimated using the Friedewald equation (Friedewald et al., 1972). Insulin was determined in singlet using an ultrasensitive insulin assay on a Beckman Coulter DXI analyser (Beckman Coulter Incorporated, USA). This analysis was performed externally by a Senior Biomedical Scientist at the Hull University Teaching Hospitals National Health Service (NHS) Trust.

Chapter 4 Prevalence of estimated non-alcoholic fatty liver disease (NAFLD) and hepatic fibrosis in self-selected, apparently healthy young to middle-aged adults using NAFLD composite biomarker panels and associations with predictors of metabolic risk

4.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterised by a spectrum of hepatocyte injury and includes two inter-related but distinct pathologies; NAFL and NASH, respectively (Chalasani et al., 2018; Marchesini et al., 2016). Fatty infiltration of the liver is a commonality of both NAFL and NASH, with the two differentiated by evidence of inflammation and hepatocyte ballooning, with or without fibrosis (Chalasani et al., 2018; Marchesini et al., 2016).

The appearance of NAFLD over recent decades has paralleled that of the co-existing obesity and T2DM epidemics, with an estimated global prevalence of 30% (Younossi et al., 2023). NAFLD has been associated with several metabolic risk factors, including: hypertension, hyperglycaemia, and dyslipidaemia (Kim et al., 2018; Younossi et al., 2016). NAFLD is therefore considered the hepatic manifestation of MetS and is prevalent in those living with clinical obesity and T2DM (Marjot et al., 2020; Younossi et al., 2016). Contemporary definitions reflective of this relationship between hepatic steatosis and metabolic dysfunction, and not simply the exclusion of alcohol excess, have been proposed (Eslam et al., 2020a; Polyzos & Mantzoros, 2020; Rinella et al., 2023). This has included recommendations for a change in the nomenclature from NAFLD to MAFLD, to more recently MASLD (Eslam et al., 2020a; Rinella et al., 2023). Traditionally, NAFL was thought to follow a benign course; whilst NASH was associated with increased liver-

related morbidity and mortality (Ekstedt et al., 2006; Teli et al., 1995a). The contemporary view is that NAFLD is dynamic and demonstrates progression as well as regression (McPherson et al., 2015; Taylor et al., 2020b). Instead, the presence and stage of hepatic fibrosis, representing the wound-healing response to liver injury, appears to be the most important histological feature to predict long-term outcomes (Angulo et al., 2015; Ekstedt et al., 2015). Hepatic fibrosis can develop in both NAFL and NASH, and may subsequently progress to cirrhosis, liver cancer, and the need for transplantation (Ekstedt et al., 2015; Younossi et al., 2016).

Early detection and staging of fibrosis in individuals living with NAFLD is therefore a priority and has necessitated the development of minimally-invasive methods to identify and determine fibrosis stage (Campos-Murguía et al., 2020). Several modalities, including hepatic imaging techniques, serum biomarkers, and composite panels have been developed (Castera et al., 2019; Tsai & Lee, 2018; Younossi et al., 2018). However, with the exception of contemporary magnetic resonance and ultrasound techniques, traditional imaging modalities are only sensitive to steatosis (Dulai et al., 2016; Esterson & Grimaldi, 2018). In addition, many serum biomarkers remain novel and are reserved to research settings due to the associated high cost and processing time (Chin et al., 2016; Jayakumar et al., 2016). Conversely, a number of composite panels as proxy indicators of hepatic fibrosis have been developed; consisting of several routinely acquired, minimally-invasive, demographic, clinical, and laboratory variables; and demonstrate good agreement with liver biopsy (Castera et al., 2019; McPherson et al., 2010; Xiao et al., 2017). These range from simple to more complex models (Table 4.1, also see section 2.4.5.5) including: AST:ALT (Williams & Hoofnagle, 1988); APRI (Wai et al., 2003); BARD score (Harrison et al., 2008); FIB-4 (Sterling et al., 2006); and NFS (Angulo et al., 2007).

Table 4.1 Summary of composite panels for the prediction of hepatic fibrosis.

Score	Items, *n*	Age	BMI	Platelet count	AST	ALT	Other components
AST:ALT	2				×	×	-
APRI	2			×	×		-
BARD score	4		×		×	×	T2DM
FIB-4	4	×		×	×	×	-
NFS	7	×	×	×	×	×	T2DM or IFG, and albumin

APRI, AST to platelet ratio index; AST:ALT, AST to ALT ratio; BARD, BMI, AST to ALT ratio and Diabetes score; FIB-4, fibrosis 4; IGF, impaired fasting glucose; NFS, NAFLD fibrosis score; T2DM, Type 2 Diabetes Mellitus. Adapted from Castera et al. (2019).

The practical advantages of composite panels allow for several potential applications, for example, integration into primary care (Armstrong et al., 2012; Tapper et al., 2016), where NAFLD is under-recognised and frequently an incidental finding (Alexander et al., 2018; Armstrong et al., 2014). This would allow for the identification and staging of hepatic fibrosis in high-risk populations and may avoid unnecessary referrals to secondary practice (Alkhouri & Feldstein, 2016; Buzzetti et al., 2015). From a research perspective, the application of minimally invasive composite panels would permit the population-based study of hepatic fibrosis and provide further endpoints to determine the efficacy of new and emerging therapies (Marchesini et al., 2016). However, there is currently no agreement as to the most appropriate composite panel for these purposes (Machado & Cortez-Pinto, 2013; Marchesini et al., 2016). Moreover, there are limited data pertaining to the use of composite panels to identify hepatic fibrosis in population-based cross-sectional studies (Kim et al., 2013; Long et al., 2016; Sesti et al., 2014).

The primary purpose of the current investigation was to therefore determine the prevalence of NAFLD and hepatic fibrosis in a self-selected cohort of apparently healthy, young to middle-aged adults undergoing routine preventive health assessment using several panels. A secondary aim of the study was to examine the cross-sectional association of lifestyle factors, predictors of metabolic risk, with estimates of hepatic steatosis and fibrosis using the above cited panels.

4.2 Methods

4.2.1 Participants

Cross-sectional data were retrospectively analysed for 510 (410 male) apparently healthy young to middle-aged adults (characteristics, mean [SD]: age: 47.1 [7.5] years; height 175.9 [8.5 cm]; body mass: 84.0 [15.9 kg]; BMI: 27.1 [4.1] $kg{\cdot}m^{-2}$). All participants underwent a Nuffield Health and Wellbeing Assessment as part of an employee benefits scheme or self-referral pathway. All participants were apparently healthy and had no prior cardiorespiratory or metabolic disorders. Data were collected between August 2006 and March 2008. Participants were asked to refrain from exercise for 24 hours and abstain from caffeine for 12 hours prior to visiting the laboratory and attended in a fasted state (≥ 12 hours postprandial). All experimental procedures were approved by the institutional ethics committee, Leeds Beckett University, Leeds, UK. All experiment procedures have previously been described in the procedures of the Nuffield cross-sectional study (Swainson et al., 2019).

The FLI, a composite score incorporating anthropometric and blood biomarkers (as detailed below), was utilised as a surrogate indicator of NAFLD. A FLI score < 10 or ≥ 60 was considered indicative of the absence or presence of NAFLD, respectively (Bedogni et al., 2006; Cuthbertson et al., 2014). Those with an intermediate FLI score (FLI 10 to 59) were also included in the analysis. For the purpose of the initial analysis,

males consuming > 21 units of alcohol per week and females > 14 units per week were excluded based on UK Government guidance at the time of data collection (Podger et al., 1995). Characteristics of all participants, including FLI subgroups and self-reported alcohol intake above recommended guidelines are summarised in Table 4.2.

4.2.2 Calculation of NAFLD composite panels

4.2.2.1 Hepatic steatosis

FLI

The FLI was calculated utilising BMI ($kg \cdot m^{-2}$), serum TGs ($mg \cdot dl^{-1}$), GGT ($IU \cdot l^{-1}$) and WC (cm) to produce a score between 0 and 100 (Bedogni et al., 2006):

$$\text{FLI} = \frac{e\,(n)}{1 + e\,(n)} \times 100$$

e = universal constant.

n $= 0.953 \times \ln(\text{TGs}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{GGT}) + 0.053 \times \text{WC} - 15.745$.

ln = natural logarithm.

4.2.2.2 Hepatic fibrosis

AST:ALT

The AST:ALT was calculated using AST ($IU \cdot l^{-1}$) and ALT ($IU \cdot l^{-1}$) according to Williams & Hoofnagle, (1988):

$$\frac{\text{AST}}{\text{ALT}}$$

Cut-offs of > 0.8 (McPherson et al., 2010) and > 1.0 (Williams & Hoofnagle, 1988) were utilised as surrogate indicators of the presence of hepatic fibrosis.

APRI

The APRI was calculated using AST ($IU \cdot l^{-1}$) and platelet count ($\times 10^{9} \cdot l^{-1}$) according to Wai et al. (2003):

$$\text{APRI} = \frac{\left(\frac{\text{AST}}{\text{ULN}}\right) \times 100}{\text{Platelet Count}}$$

ULN = upper limit of normal.

Upper limit of normal considered as 45 $IU \cdot l^{-1}$.

Cut-offs of > 0.43 and > 0.71 were utilised as surrogate indicators of the presence of any fibrosis and advanced fibrosis, respectively (Siddiqui et al., 2016).

BARD score

The BARD score was calculated according to Harrison and colleagues (2008) as the weighted sum of (**B**MI ≥ 28 $kg \cdot m^{-2}$ = 1 point, AST:ALT ≥ 0.8 = 2 points [A**AR**] and the presence of diabetes as determined by medical records or the prescription of insulin or oral hypoglycaemic medication [**D**]), to provide a composite score between 0-4. In the context of the current study, previously undiagnosed diabetes was defined according to World Health Organization & International Diabetes Federation (2006) threshold of ≥ 7.0 $mmol \cdot l^{-1}$ for fasting plasma glucose.

Advanced fibrosis was considered absent in participants with a BARD score of 0-1. A positive BARD score, that ≥ 2, was considered indicative of the presence of advanced fibrosis (Harrison et al., 2008).

FIB-4

The FIB-4 score was calculated using age (years), AST ($IU \cdot l^{-1}$), platelet count ($\times 10^{9} \cdot l^{-1}$) and ALT ($IU \cdot l^{-1}$) according to (Sterling et al., 2006):

$$\text{FIB4} = \frac{\text{Age} \times \text{AST}}{(\text{Platelet Count} \times \sqrt{\text{ALT}})}$$

Cut-offs of < 1.30 and > 2.67 were applied to discern between the absence and presence of advanced fibrosis, respectively (Anstee et al., 2019).

NAFLD Fibrosis Score

The NFS was calculated using age (years), BMI ($kg \cdot m^{-2}$), IFG ≥ 110 $mg \cdot dl^{-1}$ (6.1 $mmol \cdot l^{-1}$) or a diagnosis of diabetes, AST ($IU \cdot l^{-1}$), ALT ($IU \cdot l^{-1}$), platelet count ($\times 10^{9} \cdot l^{-1}$) and albumin ($g \cdot dl^{-1}$) according to the formula of Angulo et al. (2007):

$$\begin{aligned}\text{NFS} = {} & -1.675 + 0.037 \times \text{Age} + 0.094 \times \text{BMI} \\ & + 1.13 \times \text{IFG or Diabetes (yes} = 1, \text{No} = 0) + 0.99 \times \frac{\text{AST}}{\text{ALT}} \\ & - 0.013 \times \text{Platelet Count} - 0.66 \times \text{albumin}\end{aligned}$$

Cut-offs of < -1.455 and > 0.676 were applied to determine the absence and presence of advanced fibrosis, respectively. Composite scores between -1.455 and 0.676 were classified as indeterminate scores (Angulo et al., 2007; McPherson et al., 2010).

4.2.3 Statistical analysis

Statistical procedures were performed using IBM SPSS Statistics 23 (SPSS Incorporated, Chicago, USA). Normal (Gaussian) distribution of data was confirmed by exploratory data analysis using visual inspection of frequency histograms and quantile-quantile plots, tests for the standard error of skewness and kurtosis (-2 to +2), and the Kolmogorov-Smirnov test ($p > 0.05$) (George & Mallery, 2010). Central tendency and dispersion of the data are reported as mean and standard deviation for normally distributed data and median and quartiles 1 and 3 (Q1, Q3) for non-normally distributed data.

Comparisons of characteristics between independent groups of participants with an FLI of < 10, 10 to 59, and ≥ 60 were performed using a one-way analysis of variance (ANOVA). Outliers were identified by the visual inspection of box-plots. Homogeneity of variances was assessed by Levene's statistic and post-hoc Tukey-Kramer adjusted pairwise comparisons for unequal group sample sizes were utilised when this assumption was satisfied. When homogeneity of variances was not observed, the Welch adjusted ANOVA with post-hoc Games-Howell adjusted pairwise comparisons was used. The Kruskal-Wallis test was utilised were data violated the assumptions of the one-way ANOVA.

Distributions of median data were assessed by visual inspection of box-plots and pairwise comparisons calculated as described by Dunn (1964) with post-hoc Bonferroni correction for multiple comparisons. Categorical variables are reported as percentages (participant characteristics [Table 4.2] and the presence/absence of fibrosis or fibrosis severity [Table 4.3]) and were compared using the Chi-Square test of homogeneity with multiple z-tests of two proportions with post-hoc Bonferroni adjustment to determine between group differences. Fisher's Exact test (two-sided) was utilised if the sample size was deemed insufficient as determined by a minimum expected count of less than 5 in any cells of the

crosstab table. Multiple Fishers's Exact tests (2 x 2) with Bonferroni correction for multiple comparisons were used to determine the differences between proportions of independent groups.

Subsequent data analysis including all 510 participants (Table 4.2) was performed using structural equation modelling (SEM; Asparouhov & Muthén, 2009) in Mplus 8.4 (Muthén & Muthén, 2017), using maximum likelihood estimator. Given the anticipated relationships between factors, the oblique, goemin rotation was employed throughout. Model fit was appraised using Hu & Bentler's (1999) recommendations of the comparative fit index (CFI) and Tucker-Lewis index (TLI) of close to 0.95 for incremental indices, and the standardised root-mean-square residual (SRMR) close to 0.08 and root-mean-square error of approximation (RMSEA) of close to 0.05 were considered for absolute fit indices. Regarding standardised parameter estimates, intended factor loadings were interpreted using the recommendations of Comrey & Lee (1992) of 0.32 (poor), 0.45 (fair), 0.55 (good), 0.63 (very good), and 0.71 (excellent).

Following a theory driven approach, the observed variables of BMI, WC, systolic blood pressure (SBP), HDL inverse, TGs, glucose, ALT and GGT were utilised as predictor variables. Our initial analysis highlighted that these predictors were significantly higher in those individuals with presumed NAFLD (FLI $\geq$ 60) compared to those without NAFLD (FLI < 10, Table 4.3). Moreover, these prevalent characteristics are established risk factors for hepatic fibrosis and are included in many of the composite scoring panels (see Table 4.1). SEM enables the estimation of all standardised parameter estimates from each observed variable on to an identified number of latent variables, known as the measurement model. This approach was utilised to generate the latent variables of adiposity, MetS, and liver fat to avoid confounding between the observed variables and existing non-invasive proxy scores of NAFLD (FLI). A path from liver fat to NFS was also included in the initial hypothesised model (Table 4.5, Figure 4.1). This model was

subjected to several iterations to add or remove paths identified as theoretically appropriate or weak, or that were detrimental to model fit, respectively.

Once a satisfactory model fit was achieved by SEM, the model was further examined to determine the effect of potential moderating variables (Table 4.6 and Table 4.7). Specifically, model invariance was tested by the moderator variables of sex (male or female), smoking status (smoker or non-smoker), alcohol intake (male ≤ or > 21 units·wk^{-1}; female ≤ or > 14 units·wk^{-1}), and the number of exercise sessions per week (≤ 2 or > 2 exercise sessions·wk^{-1}) using multi-group SEM. The same four-step process for each test of invariance was followed. First, configural invariance was assessed by replicating the model across sample groups. Second, metric invariance was assessed by constraining factors. Third, scalar invariance was assessed by constraining factors and intercepts, and forth residual invariance was assessed by constraining factors, intercepts, and factor means. Model invariance was deemed to have been supported if little or no change was observed in the increasingly constrained models. Invariance was determined using Cheung & Rensvold's (2002) recommendation of $\Delta CFI \leq 0.01$ at each step.

4.3 Results

4.3.1 Participant characteristics

Characteristics of the entire cohort and by subgroup are summarised in Table 4.2. Of those participants with a self-reported alcohol consumption within Public Health England (PHE) guidelines (n = 364), 15.1% (n = 55) and 50.0% (n = 182), respectively, had a low or intermediate FLI score. Notably, 34.9% (n = 127) had an FLI score indicative of hepatic steatosis. Participants with presumed NAFLD as defined by an FLI ≥ 60 were predominantly male (90.3%). Participants with presumed NAFLD were also older and had significantly higher BMI; WC; systolic and diastolic blood pressures; prevalence of MetS; higher liver enzymes; and lower predicted $\dot{V}O_{2max}$; compared to those individuals

in which NAFLD was considered absent or who produced an intermediate score ($p < 0.001$ for all parameters respectively, Table 4.2).

Table 4.2 Participant characteristics and comparisons between FLI cut-offs.

Parameter	Entire cohort	Normal alcohol intake§	FLI < 10	FLI 10 to 59	FLI ≥ 60	p value
n	510	364	55	182	127	-
Male % (*n*)	80.40 (410)	79.40 (289)	34.55 (19)[a]	81.87 (149)[b]	95.28 (121)[c]	< 0.001^
Female % (*n*)	19.60 (100)	20.60 (75)	65.45 (36)[a]	18.13 (33)[b]	4.72 (6)[c]	< 0.001^
Age (years)	47.0 (42.0, 52.0)	47.0 (42.0, 52.0)	45.0 (40.0, 49.0)[a]	42.0 (42.0, 51.0)[a]	49.00 (44.0, 55.0)[b]	< 0.001†
Alcohol (units·wk^{-1})	12.0 (6.0, 21.25)	10.0 (4.00, 14.00)	10.0 (4.00, 12.00)[a]	10.0 (4.0,12.0)[a]	10.0 (4.0, 15.0)[a]	0.739†
Smoking % (*n*)	28.8 (147)	25.0 (91)	18.2 (10)[a]	24.18 (44)[a]	29.1 (37)[a]	0.274^
Mass (kg)	82.6 (72.5, 93.13)	82.30 (71.93, 92.80)	65.50 (60.30, 70.60)[a]	78.60 (71.98, 86.00)[b]	95.30 (88.10, 105.20)[c]	< 0.001†
BMI (kg·m^{-2})	26.44 (24.06, 29.51)	26.21 (24.02, 29.51)	22.74 (20.83, 23.94)[a]	25.28 (23.94, 26.81)[b]	30.24 (28.83, 32.56)[c]	< 0.001†
Waist (cm)	93.50 (86.34, 101.50)	93.00 (86.00, 101.00)	77.00 (74.00, 82.00)[a]	91.00 (87.00, 95.00)[b]	104.00 (99.00, 110.00)[c]	< 0.001†
WHR	0.90 (0.85, 0.94)	0.89 (0.84, 0.93)	0.78 (0.75, 0.83)[a]	0.88 (0.85, 0.92)[b]	0.94 (0.92, 0.97)[c]	< 0.001†
WHtR	0.53 (0.49, 0.57)	0.53 (0.49,0.57)	0.46 (0.44, 0.48)[a]	0.52 (0.49, 0.54)[b]	0.59 (0.56, 0.62)[c]	< 0.001†
Body fat (%)	26.10 (22.10, 31.00)	26.00 (22.00, 30.98)	26.80 (20.00, 34.90)[a]	23.50 (20.70, 27.33)[b]	28.80 (25.90, 32.00)[c]	< 0.001†
Lean mass (%)	73.90 (69.00, 77.90)	74.00 (69.03, 78.00)	73.20 (65.10, 80.00)[a]	76.50 (72.68, 79.30)[b]	71.20 (68.00, 74.10)[c]	0.001†
SBP (mmHg)	123.40 (14.61)	122.4 (14.28)	112.6 (13.01)[a]	120.5 (13.23)[b]	129.30 (12.97)[c]	< 0.001*
DBP (mmHg)	80.06 (9.33)	79.38 (9.36)	71.80 (8.80)[a]	77.81 (8.33)[b]	84.93 (7.75)[c]	< 0.001*
TC (mmol·l^{-1})	5.33 (0.93)	5.25 (0.91)	4.90 (0.71)[a]	5.13 (0.89)[a]	5.58 (0.94)[b]	< 0.001*

§Self-reported alcohol intake ≤ 14 units·wk^{-1} for women and ≤ 21 units·wk^{-1} for men. *One Way Analysis of Variance. †Kruskal Wallis H Test. ^Chi-Square test of homogeneity. Non-corresponding symbols ([a, b, c]) denote statistically significant difference between groups, exact p values reported. Data are presented as mean (standard deviation) or median (quartile 1, quartile 3).

Table 4.2 continued Participant characteristics and comparisons between FLI cut-offs.

Parameter	Entire cohort	Normal alcohol intake§	FLI < 10	FLI 10 to 59	FLI ≥ 60	p value
HDL-c ($mmol \cdot l^{-1}$)	1.40 (1.20, 1.70)	1.40 (1.20, 1.60)	1.80 (1.50, 1.90)[a]	1.50 (1.30, 1.60)[b]	1.20 (1.10, 1.40)[c]	< 0.001†
LDL-c ($mmol \cdot l^{-1}$)	3.21 (0.81)	3.17 (0.79)	2.81 (0.58)[a]	3.11 (0.76)[b]	3.42 (0.86)[c]	< 0.001*
TGs ($mmol \cdot l^{-1}$)	1.20 (0.80, 1.80)	1.20 (0.80, 1.80)	0.70 (0.60, 0.80)[a]	1.10 (0.80, 1.30)[b]	1.90 (1.40, 2.60)[c]	< 0.001†
Glucose ($mmol \cdot l^{-1}$)	5.46 (0.52)	5.43 (0.52)	5.24 (0.45)[a]	5.39 (0.50)[a]	5.57 (0.55)[b]	< 0.001*
MetS % (*n* male/female, waist ≥ 94/80 cm)	30.78 (147/10)	28.02 (94/8)	0 (0/0)[a]	12.64 (18/5)[b]	62.20 (76/3)[c]	< 0.001^
MetS % (*n* male/female, waist ≥ 102/88 cm)	23.73 (101/10)	20.10 (65/8)	0 (0/0)[a]	5.49 (5/5)[a]	49.61 (60/3)[b]	< 0.001^
AST ($U \cdot l^{-1}$)	33.00 (27.0, 40.0)	33.00 (27.00, 40.00)	29.00 (25.0, 34.0)[a]	32.00 (27.00, 40.00)[a]	35.00 (30.00, 42.00)[b]	< 0.001†
ALT ($U \cdot l^{-1}$)	27.00 (21.00, 36.00)	27.00 (21.00, 35.00)	17.0 (14.0, 21.0)[a]	26.00 (21.00, 31.00)[b]	35.00 (28.00, 49.00)[c]	< 0.001†
GGT ($U \cdot l^{-1}$)	22.00 (13.00, 37.00)	21.00 (13.00, 31.00)	10.0 (6.0, 12.0)[a]	18.00 (13.00, 26.00)[b]	31.00 (25.00, 49.00)[c]	< 0.001†
Predicted $\dot{V}O_{2max}$	42.42 (35.52, 51.40)	42.42 (35.59, 50.65)	48.11 (42.42, 52.81)[a]	43.88 (36.73, 52.40)[a]	38.13 (31.45, 43.22)[b]	< 0.001†

§Self-reported alcohol intake ≤ 14 $units \cdot wk^{-1}$ for women and ≤ 21 $units \cdot wk^{-1}$ for men. *One Way Analysis of Variance. †Kruskal Wallis H Test. ^Chi-Square test of homogeneity. Non-corresponding symbols ($^{a, b, c}$) denote statistically significant difference between groups, exact p values reported. Data are presented as mean (standard deviation) or median (quartile 1, quartile 3).

4.3.2 Prevalence of hepatic fibrosis

The prevalence of hepatic fibrosis as determined by the composite panels, including the AST:ALT, APRI, BARD, FIB-4, and NFS, are detailed in Table 4.3. For the AST:ALT threshold of 0.8, there was a significantly higher number of cases without NAFLD and with an intermediate score predicted to have advanced fibrosis compared to those with presumed NAFLD (100%, 97.25% and 75.59%, respectively, $p = 0.001$, Table 4.3). For the higher binary cut-off of 1.0, the predicted prevalence of advanced fibrosis was significantly higher in those cases where NAFLD was considered absent, in comparison to those generating an intermediate score and with presumed NAFLD (98.20%, 80.77%, and 45.67%, correspondingly, $p < 0.001$, Table 4.3).

The presence of fibrosis as indicated by the 0.43 cut-off for the APRI was significantly higher in those with NAFLD compared to those without (28.35% and 10.91%, $p = 0.038$). However, the prevalence of advanced fibrosis did not differ between groups as categorised by the higher 0.71 APRI threshold (1.82% and 0.79%, $p = 0.639$).

For BARD, advanced fibrosis was considered absent in a significantly higher number of those with presumed NAFLD than without (22.83% and 0%, $p < 0.001$); as indicated by a score of 0-1. Conversely, advanced fibrosis was predicted to be significantly higher in those cases where NAFLD was considered absent, compared to presumed (100.00% and 51.18%, $p < 0.001$).

There were no significant differences in the number of cases in which advanced fibrosis was ruled in (0.00% and 3.40%, $p = 0.109$), ruled out (51.18% and 49.10%, $p = 0.962$), or considered inconclusive (48.82% and 47.30%, $p = 0.934$), for those with and without NAFLD, respectively, as predicted by the FIB-4.

Finally, for the NFS, the risk of advanced fibrosis was predicted to low (49.60% and 65.50%, $p = 0.026$), intermediate (48.32% and 32.73%, $p = 0.035$), or high (1.57% and 1.82%, $p = 0.048$) in volunteers where NAFLD was considered present and absent, correspondingly.

Table 4.3 Composite scores of hepatic fibrosis and comparisons between FLI cut-offs.

Composite score	Entire cohort	Normal alcohol intake§	FLI < 10	FLI 10 to 59	FLI ≥ 60	p value
n	510	364	55	182	127	-
AST/ALT Ratio > 0.8 % (*n*)	89.41(456)	90.11 (328)	100 (55)[a]	97.25 (177)[a]	75.59 (96)[b]	< 0.001^
AST/ALT Ratio > 1.0 % (*n*)	70.39 (359)	71.15 (259)	98.20 (54)[a]	80.77 (147)[b]	45.67 (58)[c]	< 0.0001^
APRI > 0.43 % (*n*)	21.96 (112)	23.35 (85)	10.91 (6)[a]	23.63 (43)[a, b]	28.35 (36)[b]	0.038^
APRI > 0.71 % (*n*)	1.76 (9)	1.65 (6)	1.82 (1)[a]	2.20 (4)[a]	0.79 (1)[a]	0.639‡
BARD 0-1 % (*n*)	10.20 (52)	9.34 (34)	0 (0)[a]	2.75 (5)[a]	22.83 (29)[b]	< 0 .001^
BARD 2-4 % (*n*)	89.80 (458)	90.66 (330)	100 (55)[a]	97.25 (177)[a]	77.17 (98)[b]	< 0.001^
FIB 4 < 1.30 % (*n*)	51.96 (265)	50.82 (185)	49.10 (27)[a]	51.10 (93)[a]	51.18 (65)[a]	0.962^
FIB 4 1.30-2.67 % (*n*)	46.27 (236)	47.53 (173)	47.30 (26)[a]	46.70 (85)[a]	48.82 (62)[a]	0.934^
FIB 4 > 2.67 % (*n*)	1.76 (9)	1.65 (6)	3.64 (2)[a]	2.20 (4)[a]	0 (0)[a]	0.109‡
NFS < -1.455 % (*n*)	58.63 (299)	59.10 (215)	65.50 (36)[a]	63.74 (116)[a]	49.60 (63)[b]	0.026^
NFS -1.455 to 0.676 % (*n*)	40.78 (208)	39.84 (145)	32.73 (18)[a]	35.71 (65)[a]	48.82 (62)[b]	0.035^
NFS > 0.676 % (*n*)	0.98 (5)	1.10 (4)	1.82 (1)[a]	0.55 (1)[a]	1.57 (2)[a]	0.482‡

§Self-reported alcohol intake ≤ 14 units·wk^{-1} for women and ≤ 21 units·wk^{-1} for men. ^Chi-Square test of homogeneity. ‡Fishers Exact test. Non-corresponding symbols ([a, b, c]) denote statistically significant difference between groups, exact p values reported. Data are presented as mean (standard deviation) or median (quartile 1, quartile 3).

4.3.3 SEM and invariance testing

Each model was evaluated and modified through an iterative process. The initial hypothesised model presented an unsatisfactory fit (Chi squared [χ^2] = 268.934 [degrees of freedom, 23], CFI = 0.854, $p < 0.001$, TLI = 0.772, SRMR = 0.094, RMSEA [90% confidence interval] = 0.145 [0.130, 0.161], SEM 1, Table 4.4, Figure 4.1). Inspection of modification indices indicated an improvement in model fit with the inclusion of a path from adiposity to NFS. The addition of this path was theoretically appropriate because of the observed prevalence of obesity in individuals diagnosed with NAFLD/NASH (Younossi et al., 2016) and was therefore included in model 2 (Table 4.5, SEM2).

Model fit was improved following this iteration (χ^2 = 180.027 [22], $p < 0.001$, CFI = 0.906, TLI = 0.847, SRMR = 0.065, RMSEA [90% CI] = 0.119 [0.103, 0.135], Table 4.5, SEM2). Following a review of model 2, a decision was made to remove the path from adiposity to liver fat. The rationale for this step was twofold; first, to reflect the proportion of unique variance explained by the inclusion of the path from adiposity to NFS (standardised parameter estimate [95% CI] = 0.46 [0.37, 0.55], $p < 0.001$, Table 4.5, SEM2); and second, the subsumed variance in the path from adiposity to liver fat (standardised parameter estimate [95% CI] = 0.13 [0.00, 0.26], $p = 0.06$, Table 4.5, SEM 2). Adiposity therefore accounted for a fair proportion of the variance in NFS; however, it was considered a poor predictor of liver fat.

An appraisal of model 3 demonstrated similar fit indices to the previous iteration (χ^2 = 183.527 [23], $p < 0.001$, CFI = 0.905, TLI = 0.851, SRMR = 0.065, RMSEA [90% CI] = 0.117 [0.102, 0.133], Table 4.5, SEM 3), although all model paths were now statistically significant ($p < 0.001$ respectively, Table 4.5, SEM 3). A review of the path loadings highlighted that MetS accounted for a greater proportion of the variance in liver fat (standardised parameter estimates

[95% CI] = 0.63 [0.54, 0.72], p < 0.001, Table 4.5, SEM 3) following the removal of the path from adiposity to liver fat. The proportion of this variance appeared to be unique to MetS as the magnitude of the loadings of the paths from adiposity to NFS, and liver fat to NFS, remained fair (standardised parameter estimate [95% CI] = 0.45 [0.36, 0.54], p < 0.001) and poor (standardised parameter estimate [95% CI] = -0.30 [-0.40, -0.20], p < 0.001), respectively (Table 4.5, SEM 3). Evaluation of modification indices indicated that a further iteration to include a path between TGs and HDL inverse would improve model fit. As both TGs and HDL inverse are predictors of MetS allowing their errors to correlate given their relationship was justified.

Inclusion of the path between TGs to HDL inverse demonstrated a statistically significant relationship between the two predictors (standardised parameter estimate [95% CI] = 0.33 [0.23, 0.42], p < 0.001, Table 4.5, Figure 4.2), and subsequently improved fit indices for model 4 (χ^2 = 149.194 [22], p < 0.001, CFI = 0.925, TLI = 0.876, SRMR = 0.054, RMSEA [90% CI] = 0.106 [0.091, 0.123], Table 4.5, Figure 4.2). A review of the modification index suggested an additional path between HDL inverse and SBP would further reduce the Chi Squared value. Both observed variables are indicators of MetS; therefore, allowing their errors to correlate was appropriate.

The inclusion of the path between HDL inverse and SBP caused model fit to deteriorate (χ^2 = 288.330 [21], p < 0.001, CFI = 0.841, TLI = 0.728, SRMR = 0.084, RMSEA [90% CI] = 0.158 [0.142, 0.174], Table 4.5, SEM 5). Model 4 was therefore selected as the final iteration (Table 4.5 SEM 4, Figure 4.2). Although the fit indices for model 4 were equivocal, the measurement model and subsequent paths represented a theoretically sound approach to analysing the data; as confirmed by all path loadings being statistically significant (p < 0.001 respectively, Table 4.5, Figure 4.2). The modification indices suggested the misspecifications resulting in

equivocal model fit could be attributed to the covariance between predictors; for example, WC as an indicator of both adiposity and MetS.

Model 4 was further examined to determine if the observed variables of sex, smoking status, physical activity, and alcohol intake were invariant across the model. When the measurement model is constrained to be equal across the groups, the structural paths in the model are freely estimated. Acceptable model fit indicates invariance, or no difference, between groups. Model fit for sex, smoking status, and physical activity remained invariant for the configural, metric, and scalar iterations (Table 4.6). However, model fit to test residual invariance deteriorated and was unsatisfactory for sex (χ^2 = 432.289 [59], p < 0.001, CFI = 0.756, TLI = 0.703, SRMR = 0.369, RMSEA [90% CI] = 0.158 [0.144, 0.172], Table 4.6); smoking status (χ^2 = 723.36 [59], p < 0.001, CFI = 0.604, TLI = 0.517, SRMR = 0.392, RMSEA [90% CI] = 0.210 [0.197, 0.224], Table 4.6), and physical activity (χ^2 = 553.673 [59], p < 0.001, CFI = 0.706, TLI = 0.641, SRMR = 0.344, RMSEA [90% CI] = 0.181 [0.168, 0.195], Table 4.6), correspondingly. Therefore, the aforementioned models remained invariant when replicated with factors and item intercepts constrained, meaning that sex, smoking status, and physical activity had limited influence on path loadings; with all structural paths, except that from TGs to HDL inverse in females (standardised parameter estimate [95% CI] = 0.126 [-0.94, 0.345], p = 0.261), remaining statistically significant. Standardised parameters estimates are presented in Table 4.7.

Regarding alcohol intake, the configural and metric models were invariant (Table 4.6). There was, however, an undesirable reduction in CFI following the scalar iteration (χ^2 = 220.299 [55], p < 0.001, CFI = 0.903, TLI = 0.873, SRMR = 0.073, RMSEA [90% CI] = 0.109 [0.094, 0.124], Table 4.7). Although the change in CFI was > 0.01, the model fit was considered equivocal with all path loadings remaining statistically significant across the two groups (Table

4.7). The final model to test residual invariance demonstrated an unacceptable reduction in both CFI and model fit (χ^2 = 448.115 [59], $p < 0.001$, CFI = 0.772, TLI = 0.721, SRMR = 0.258, RMSEA [90% CI] = 0.161 [0.147, 0.175], Table 4.6). Alcohol intake therefore had little effect on the path loadings, with all structural paths remaining statistically significant following the tests for configural, metric, and scalar invariance, respectively.

Table 4.4 Model fit for SEM models 1,2,3,4 & 5.

Model	χ^2	df	CFI	TLI	SRMR	RMSEA (90% CI)
SEM 1	268.934*	23	0.854	0.772	0.094	0.145 (0.130, 0.161)
SEM 2	180.027*	22	0.906	0.847	0.065	0.119 (0.103, 0.135)
SEM 3	183.527*	23	0.905	0.851	0.065	0.117 (0.102 ,0.133)
SEM 4	149.194*	22	0.925	0.876	0.054	0.106 (0.091, 0.123)
SEM 5	288.330*	21	0.841	0.728	0.084	0.158 (0.142, 0.174)

SEM = structural equation model. χ^2 = Chi squared, df = degrees of freedom, CFI = comparative fit index, TLI = Tucker-Lewis index, SRMR = standardised root-mean-squared residual, RMSEA = root-mean-square error of approximation, 90% CI = 90% confidence interval, *denotes statistically significant difference ($p < 0.001$).

Table 4.5 Standardised parameter estimates (95% CI) for SEM models 1 2, 3, 4 and 5.

Model	Standardised parameter estimates (95% CI)														
	WC →Adi	BMI → Adi	SBP →Met	HDL→MetS	TGs →MetS	Glu→MetS	WC→MetS	ALT→LF	GGT→LF	MetS → LF	Adi → LF	LF → NFS	-	Met ↔ Adi	-	-
SEM 1	0.88 (0.57, 1.20)*	0.92 (0.78, 1.05)*	0.42 (0.33, 0.51)*	0.64 (0.57, 0.72)*	0.71 (0.63, 0.79)*	0.27 (0.17, 0.36)*	0.12 (-0.18, 0.41)	0.89 (0.79, 0.98)*	0.63 (0.55, 0.72)*	0.51 (0.35, 0.66)*	0.16 (0.01, 0.31)*	-0.06 (-0.17, 0.05)		0.62 (0.48, 0.76)*		
	WC →Adi	BMI → Adi	SBP →Met	HDL→MetS	TGs →MetS	Glu→MetS	WC→MetS	ALT→LF	GGT→LF	MetS → LF	Adi → LF	LF → NFS	Adi →NFS	Met ↔ Adi		
SEM 2	0.74 (0.64, 0.84)*	0.99 (0.94, 1.04)	0.42 (0.33, 0.51)*	0.64 (0.57, 0.72)*	0.70 (0.63, 0.78)*	0.27 (0.17, 0.36)*	0.25 (0.14, 0.35)*	0.90 (0.82, 0.98)*	0.62 (0.54, 0.70)*	0.53 (0.40, 0.67)*	0.13 (-0.003, 0.26)	-0.31 (-0.41, -0.21)*	0.46 (0.37, 0.55)*	0.58 (0.49, 0.67)*	-	-
	WC →Adi	BMI → Adi	SBP →Met	HDL→MetS	TGs →MetS	Glu→MetS	WC→MetS	ALT→LF	GGT→LF	MetS → LF	-	LF → NFS	Adi → NFS	Met ↔ Adi	-	-
SEM 3	0.72 (0.62, 0.82)	0.99 (0.94, 1.04)	0.43 (0.35, 0.52)	0.63 (0.55, 0.70)	0.68 (0.61, 0.75)	0.27 (0.18, 0.36)	0.26 (0.16, 0.37)	0.91 (0.83, 0.99)	0.61 (0.54, 0.69)	0.63 (0.54, 0.72)	-	-0.30 (-0.40, -0.20	0.45 (0.36, 0.54)	0.62 (0.53, 0.70)	-	-
	WC →Adi	BMI → Adi	SBP →Met	HDL→MetS	TGs →MetS	Glu→MetS	WC→MetS	ALT→LF	GGT→LF	MetS → LF	-	LF → NFS	Adi → NFS	Met ↔ Adi	TGs ↔ HDL	
SEM 4	0.65 (0.52, 0.78)*	0.99 (0.94, 1.04)*	0.48 (0.39, 0.56)*	0.52 (0.44, 0.60)*	0.56 (0.48, 0.65)*	0.27 (0.19, 0.38)*	0.33 (0.20, 0.47)*	0.88 (0.80, 0.96)*	0.64 (0.56, 0.71)*	0.64 (0.55, 0.74)*	-	-0.31 (-0.42, -0.21)*	0.47 (0.37, 0.56)*	0.71 (0.62, 0.80)*	0.33 (0.23, 0.42)*	
	WC → Adi	BMI →Adi	SBP →Met	HDL →MetS	TGs →MetS	Glu →MeSt	WC→MetS	ALT→LF	GGT→LF	MetS → LF	-	LF → NFS	Adi → NFS	Met ↔ Adi	TGs ↔ HDL	HDL ↔ SBP
SEM 5	3.75 (-6.26, .13.76)	0.83 (0.70, 0.96)*	0.46 (0.37, 0.55)*	0.45 (.36, .54)*	0.48 (0.37, 0.59)*	0.37 (0.25, .043)*	-2.78 (-12.77, 7.22)	0.43 (0.35, 0.51)*	0.30 (0.21, .040)*	1.21 (1.02, 1.41)*	-	0.95 (-0.29, 2.19)	-0.73 (-2.1, 6.2)	0.97 (0.92, 1.03)*	0.40 (0.31, 0.49)*	-0.14 (-0.22, -0.05)*

Adi = adiposity, ALT = alanine amino transferase, BMI = body mass index, GGT = gamma-glutamyl transferase, Glu = glucose, HDL = high density lipoprotein, LF = liver fat, MetS = metabolic syndrome, NFS = NAFLD fibrosis score, SBP = systolic blood pressure, TGs = triglycerides, WC = waist circumference. *Denotes statistically significant difference ($p < 0.05$).

Table 4.6 Invariance testing for sex, smoking status, alcohol intake and physical activity.

Model	χ^2	df	$\Delta \chi^2$	Δdf	CFI	ΔCFI	TLI	SRMR	RMSEA (90% CI)
Sex									
Configural invariance	170.177*	44	-	-	0.918	-	0.865	0.062	0.106 (0.089, 0.123)
Metric invariance	183.156*	50	12.979	6	0.913	0.005	0.875	0.069	0.102 (0.087, 0.118)
Scalar invariance	194.495*	55	11.339	5	0.909	0.004	.0881	0.077	0.100 (0.085, 0.115)
Residual invariance	432.289*	59	237.794	4	0.756	0.153	0.703	0.369	0.158 (0.144, 0.172)
Smoking status									
Configural invariance	182.716*	44	-	-	0.917	-	0.8650	.058	0.111 (.095, .128)
Metric invariance	185.630*	50	2.914	6	0.919	0.002	0.884	0.062	0.103 (0.088, 0.119)
Scalar invariance	193.049*	55	7.419	5	0.918	0.001	0.892	0.065	0.099 (0.084, 0.115)
Residual invariance	723.364*	59	530.315	4	0.604	0.314	0.517	0.392	0.210 (0.197, 0.224)

χ^2 = Chi squared, df = degrees of freedom, $\Delta \chi^2$ = change in Chi squared, Δdf = change in degrees of freedom, CFI = comparative fit index, ΔCFI = change in comparative fit index, TLI = Tucker-Lewis index, SRMR = standardised root-mean-squared residual, RMSEA = root-mean-square error of approximation, 90% CI = 90% confidence interval, *denotes statistically significant difference ($p < 0.001$).

Table 4.6 continued Invariance testing for sex, smoking status, alcohol intake and physical activity.

Model	χ^2	df	$\Delta \chi^2$	Δdf	CFI	ΔCFI	TLI	SRMR	RMSEA (90% CI)
Alcohol intake									
Configural invariance	173.498*	44	-	-	0.924	-	0.876	0.059	0.107 (0.091, 0.124)
Metric invariance	178.214*	50	4.716	6	0.925	0.001	0.892	0.065	0.100 (0.085, 0.116)
Scalar invariance	220.299*	55	42.085	5	0.903	0.022	0.873	0.073	0.109 (0.094, 0.124)
Residual invariance	448.115*	59	227.816	4	0.772	0.131	0.721	0.258	0.161 (0.147, 0.175)
Physical activity									
Configural invariance	174.589*	44	-	-	0.922	-	0.873	0.058	0.108 (0.091, 0.125)
Metric invariance	181.030*	50	6.441	6	0.922	0.000	0.888	0.068	0.101 (0.086, 0.117)
Scalar invariance	188.929*	55	7.899	5	0.920	0.002	0.896	0.068	0.098 (0.083, 0.113)
Residual invariance	553.673*	59	364.744	4	0.706	0.214	0.641	0.344	0.181 (0.168, 0.295)

χ^2 = Chi squared, df = degrees of freedom, $\Delta \chi^2$ = change in Chi squared, Δdf = change in degrees of freedom, CFI = comparative fit index, ΔCFI = change in comparative fit index, TLI = Tucker-Lewis index, SRMR = standardised root-mean-squared residual, RMSEA = root-mean-square error of approximation, 90% CI = 90% confidence interval, *denotes statistically significant difference ($p < 0.001$).

Table 4.7 Standardised parameter estimates (95% CI) for invariance testing (scalar models) for sex, smoking status, alcohol intake, and physical activity.

Model	Standardised parameter estimates (95% CI)													
Sex	WC → Adi	BMI →Adi	SBP →MetS	HDL →MetS	TGs →MetS	Glu →MetS	WC→MetS	ALT→LF	GGT→LF	MetS → LF	LF → NFS	Adi → NFS	MetS ↔ Adi	TGs ↔ HDL
Male	0.47 (0.35, 0.59)*	1.00 (0.95, 1.05)*	0.34 (0.27, 0.42)*	0.38 (0.31, 0.45)*	0.33 (0.27, 0.40)*	0.21 (0.14, .028)*	0.48 (0.36, 0.60)*	0.85 (0.76, 0.93)*	0.60 0(.53, 0.67)*	0.48 (0.34, 0.56)*	-0.31 (-0.42, -0.20)*	0.44 (0.33, 0.54)*	0.89 (0.79, 1.00)*	0.41 (0.32, 0.49)*
Female	0.55 0(.43, 0.68)*	1.04 (0.93, 1.16)*	0.32 (0.22, 0.41)*	0.45 (0.34, 0.56)*	0.56 (0.45, 0.68)*	0.21 (0.13, 0.30)*	0.47 (0.34, 0.60)*	0.94 (0.81, 1.07)*	0.59 (0.48, 0.69)*	0.58 (0.37, 0.79)*	-0.33 (-0.53, -0.14)*	0.44 (0.26, 0.63)*	0.50 (0.27, 0.73)*	0.13 (-0.09, 0.35)
Smoking status	WC → Adi	BMI →Adi	SBP →MetS	HDL →MetS	TGs →MetS	Glu →MetS	WC→MetS	ALT→LF	GGT→LF	MetS → LF	LF → NFS	Adi → NFS	MetS ↔ Adi	TGs ↔ HDL
Smoker	0.64 (0.52, 0.77)*	0.99 (0.92, 1.06)*	0.50 (0.39, 0.62)	0.51 (0.40, 0.62)	0.52 (0.40, 0.64)*	0.30 (0.19, 0.41)*	0.34 (0.20, 0.47)*	0.91 (0.81, 1.01)*	0.69 (0.59, 0.78)*	0.66 (0.51, 0.82)*	-0.28 (-0.47, -0.10)*	0.38 (0.21, 0.55)*	0.70 (0.54, 0.87)*	0.29 (0.12, 0.46)*
Non-smoker	0.65 (0.51, 0.78)*	0.98 (0.94, 1.03)*	0.45 (0.36, 0.53)*	0.52 (0.43, 0.61)*	0.57 (0.49, 0.66)*	0.28 (0.19, 0.37)*	0.33 (0.19, 0.47)*	0.87 (0.78, 0.96)*	0.61 (0.53, 0.69)*	0.63 (0.51, 0.74)*	-0.33 (-0.46, -0.21)*	0.51 (0.40, 0.62)*	0.72 (0.61, 0.82)*	0.34 (0.23, 0.44)*

Adi = adiposity, ALT = alanine amino transferase, BMI = body mass index, GGT = gamma-glutamyl transferase, Glu = glucose, HDL = high density lipoprotein, LF = liver fat, MetS = metabolic syndrome, NFS = NAFLD fibrosis score, SBP = systolic blood pressure, TGs = triglycerides, WC = waist circumference. *Denotes statistically significant difference ($p < 0.05$).

Table 4.7 continued Standardised parameter estimates (95% CI) for invariance testing (scalar models) for sex, smoking status, alcohol intake, and physical activity.

Model	Standardised parameter estimates (95% CI)												
Alcohol intake	WC → Adi	BMI →Adi	SBP →MetS	HDL →MetS	TGs →MetS	Glu →MetS	WC→MetS	ALT→LF	GGT→LF	MetS → LF	LF → NFS	Adi → NFS	MetS ↔ Adi	TGs ↔ HDL
Moderate	0.63 (0.50, 0.77)*	1.00 (0.95, 1.05)*	0.47 (0.38, 0.55)*	0.51 (0.42, 0.60)*	0.54 (0.45, 0.64)*	0.27 (0.18, 0.36)*	0.34 (0.20, 0.47)*	0.89 (0.80, 0.98)*	0.67 (0.59, 0.75)*	0.60 (0.49, 0.72)*	-0.26 (-0.38, -0.14)*	0.45 (0.34, 0.56)*	0.73 (0.62, 0.84)*	0.31 (0.20, 0.42)*
Excessive	0.61 (0.46, 0.75)*	0.97 (0.91, 1.04)*	0.50 (0.39, 0.61)*	0.56 (0.46, 0.66)*	0.57 (0.46, 0.68)*	0.30 (0.20, 0.41)*	0.40 (0.24, 0.55)*	0.90 (0.79, 1.00)*	0.56 (0.46, 0.66)*	0.68 (0.53, 0.83)*	-0.44 (-0.63, -0.25)*	0.51 (0.33, 0.69)*	0.70 (0.54, 0.86)*	0.41 (0.26, 0.55)*
Physical activity	WC → Adi	BMI →Adi	SBP →MetS	HDL →MetS	TGs →MetS	Glu →MetS	WC→MetS	ALT→LF	GGT→LF	MetS → LF	LF → NFS	Adi → NFS	MetS ↔ Adi	TGs ↔ HDL
Sedentary	0.63 (0.49, 0.77)*	0.96 (0.91, 1.01)*	0.52 (0.43, 0.61)*	0.49 (0.40, 0.59)*	0.56 (0.46, 0.67)*	0.29 (0.19, 0.39)*	0.37 (0.22, 0.52)*	0.85 (0.76, 0.95)*	0.64 (0.55, 0.72)*	0.61 (0.49, 0.74)*	-0.30 (-.044, -0.16)*	0.50 (0.38, 0.63)*	0.71 (0.58, 0.84)	0.41 (0.30, 0.53)*
Active	0.62 (0.49, 0.76)*	1.03 (0.97, 1.09)*	0.42 (0.33, 0.52)*	0.52 (0.43, 0.62)*	0.53 (0.43, 0.62)*	0.26 (0.16, 0.35)*	0.33 (0.19, 0.47)*	0.89 (0.81, 0.98)*	0.66 (0.57, 0.75)*	0.66 (0.52, 0.79)*	-.036 (-0.51, -0.22)*	.44 (0.30, 0.57)*	0.72 (0.60, 0.84)*	0.21 (0.08, 0.35)

Adi = adiposity, ALT = alanine amino transferase, BMI = body mass index, GGT = gamma-glutamyl transferase, Glu = glucose, HDL = high density lipoprotein, LF = liver fat, MetS = metabolic syndrome, NFS = NAFLD fibrosis score, SBP = systolic blood pressure, TGs = triglycerides, WC = waist circumference. *Denotes statistically significant difference ($p < 0.05$).

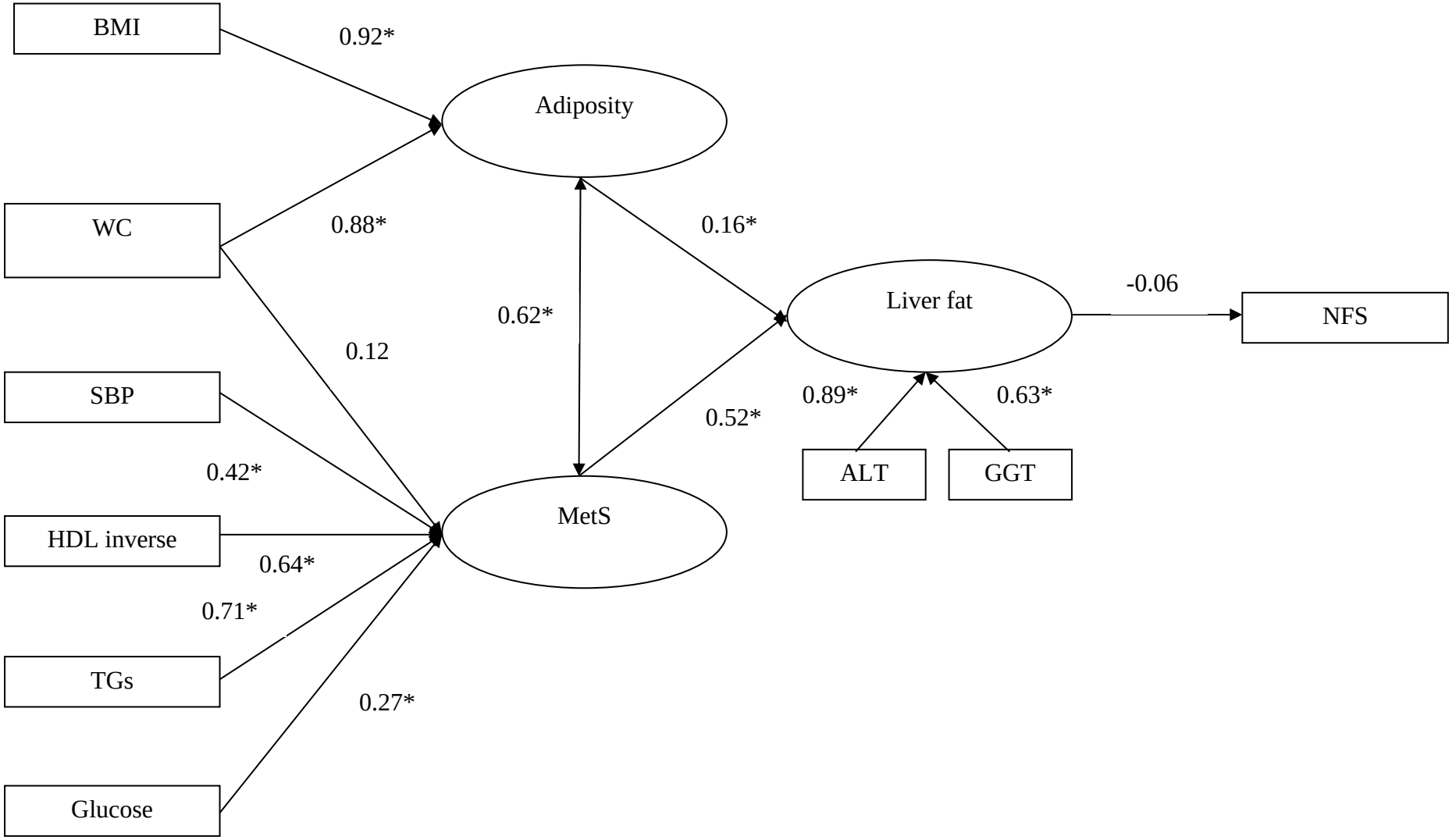

Figure 4.1 Initial hypothesised model. SEM 1: χ^2 (23) = 268.934, p < 0.001, CFI = 0.854, TLI = 0.772, SRMR = 0.094, RMSEA (90% CI) = 0.145 (0.130, 0.161). ALT, alanine aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; MetS, metabolic syndrome; NFS, non-alcoholic fatty liver disease fibrosis score; SBP, systolic blood pressure; TGs, triglycerides; WC, waist circumference. *****Statistically significant p < 0.001.

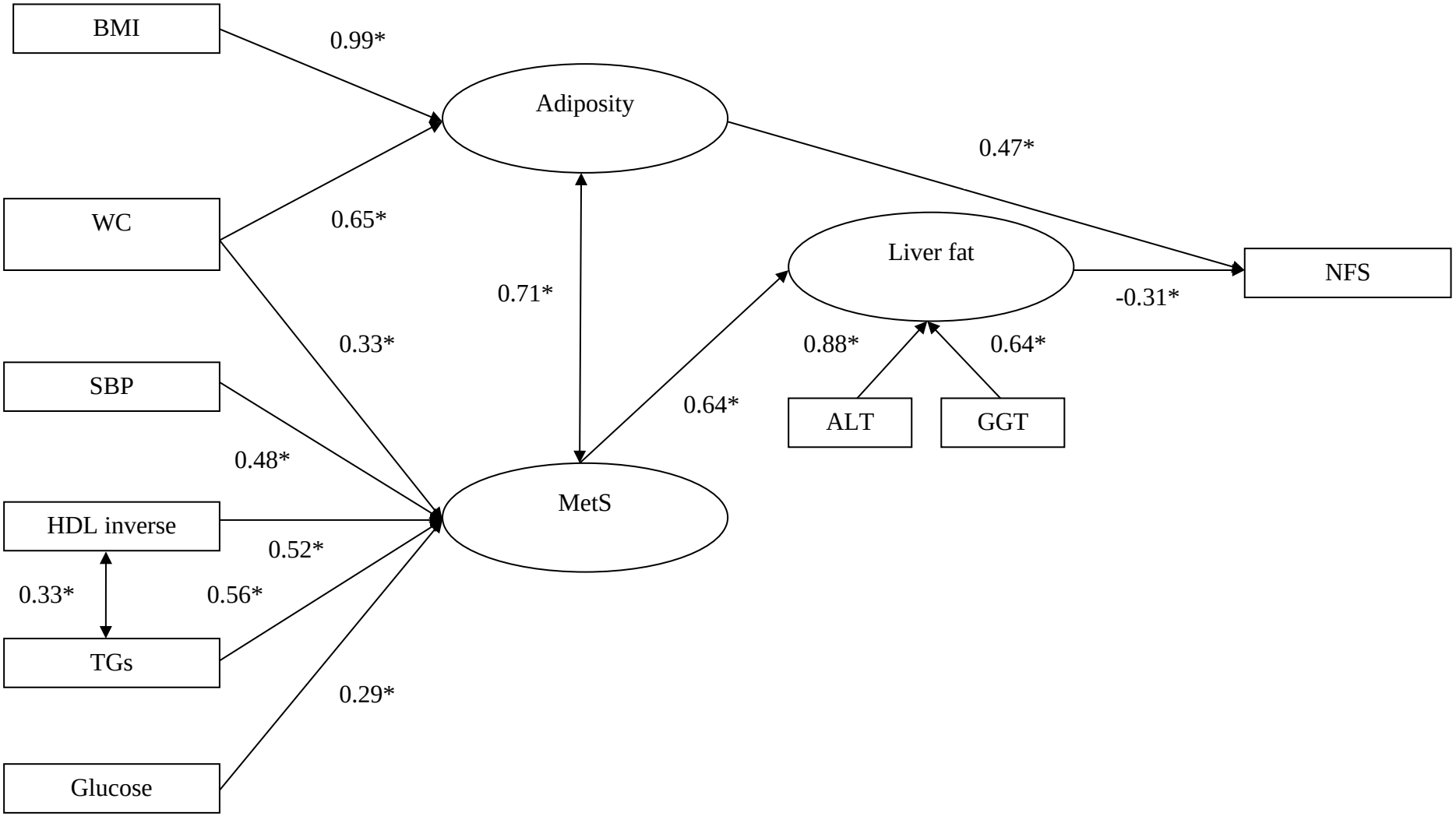

Figure 4.2 Final model. SEM 4: χ^2 (22) = 149.194, p < 0.001, CFI = 0.925, TLI = 0.876, SRMR = 0.054, RMSEA (90% CI) = 0.106 (0.091, 0.123). ALT, alanine aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; MetS, metabolic syndrome; NFS, non-alcoholic fatty liver disease fibrosis score; SBP, systolic blood pressure; TGs, triglycerides; WC, waist circumference. *****Statistically significant p < 0.001.

4.4 Discussion

The primary aim of the current study was to determine the prevalence of NAFLD and risk of hepatic fibrosis as predicted by non-invasive composite panels in a self-selected cohort of apparently healthy, young to middle-aged adults attending for a preventive health assessment. The secondary purpose of the investigation was to explore the association between predictors of metabolic risk, adiposity, MetS and composite scores of NAFLD and hepatic fibrosis within the same sample.

4.4.1 Prevalence of NAFLD

The predicted prevalence of NAFLD in the current cohort was 34.9% as determined by the FLI (Bedogni et al., 2006). This included participants with self-reported weekly alcohol consumption within PHE guidelines at the time of data collection (Podger et al., 1995). Previous estimates suggest that between 17-33% of the UK adult population are living with NAFLD (Abeysekera et al., 2020; Armstrong et al., 2012; Williams et al., 2014); similar to that of other European countries (Blachier et al., 2013; Younossi et al., 2016, 2023). Overall, the worldwide prevalence of NAFLD is estimated to be approximately 30%, with the highest number of cases observed in Latin America and the Middle East (Younossi et al., 2023). Although comparable, the discrepancies between these estimates are perhaps not surprising given the different methodologies and cohorts utilised to determine the prevalence of NAFLD (Younossi et al., 2016, 2023). Typically, cross-sectional population-based studies using ultrasound as the diagnostic technique to determine hepatic steatosis have been reported (Le et al., 2022; Younossi et al., 2016, 2023). However, several other study designs, including: case-control, case-series and longitudinal studies; different samples, such as referrals to secondary care or hospitalisations; and diagnostic methods, for example: blood markers, imaging, liver

biopsy, and/or a combination of these techniques, have also been used (Le et al., 2022; Younossi et al., 2016, 2023). Given these complexities, drawing direct comparisons between these studies is therefore challenging.

In the current study, the FLI comprising of WC, BMI, TGs and GGT, was used to determine the prevalence of hepatic steatosis (Bedogni et al., 2006). Initially validated against ultrasound within a Caucasian population, the FLI has since received external validation when compared to ^{1}H-MRS within apparently healthy controls and obese, insulin-resistant individuals, respectively (Cuthbertson et al., 2014). Although the FLI was not able to quantify liver fat content in percentage terms, it was able to accurately determine the presence or absence of hepatic steatosis based on the cut-offs applied in this chapter. For example, an FLI $\geq$ 10 had a sensitivity of 95% and a negative likelihood ratio of 0.15; meaning an individual without hepatic steatosis is approximately seven times more likely to have an FLI $\geq$ 10 (Cuthbertson et al., 2014). Moreover, an FLI $\geq$ 60 gave a specificity of 91% and a positive likelihood ratio of 5.10; therefore an individual with hepatic steatosis is approximately five times more likely to have an FLI $\geq$ 60 (Cuthbertson et al., 2014). The FLI may therefore be used for the purposes of screening in clinical practice and research settings (Cuthbertson et al., 2014). It was for these reasons that the FLI was preferred to other composite panels of predicting hepatic steatosis within the current cohort and to similarly maintain a thread by which to characterise/identify participants for subsequent studies included in this thesis.

A more recent meta-analysis of global NAFLD prevalence reported that composite panels, including the FLI, were utilised as the primary diagnostic method in approximately 10% of studies cited, with an estimated NAFLD prevalence of 25.6% (Le et al., 2022). Of these, six were from European countries of which 28.7% of participants had an FLI $\geq$ 60 (Byambasukh et al., 2019; Croci et al., 2019; Kanerva et al., 2014; Rietman et al., 2018; van den Berg et al., 2017, 2019); comparable to our estimate of 34.9% within a UK cohort.

4.4.2 Prevalence of hepatic fibrosis

The predicted prevalence of hepatic fibrosis varied significantly according to the complexity of the composite scoring panel and cut-off applied to discern between the risk of, or absence and presence of advanced fibrosis. For example, in those participants with presumed NAFLD, the prevalence of advanced fibrosis was predicted to be 0%, 0.79%, 1.57%, 45.67%, and 77.17% for the FIB-4, APRI, NFS, AST:ALT, and BARD, respectively (Table 4.3). Similar findings were documented by Long and colleagues (2016) who also observed widely disparate predictions of hepatic fibrosis within a sample taken from the Framingham Heart Study (Dawber et al., 1951). Following exclusion criteria, including alcohol excess, 1968 Offspring, Third Generation and Omni 2 Cohort participants who also volunteered for the multi-detector CT 2 sub-study for the determination of ectopic fat, including liver fat, between September 2008 and December 2011 were sampled (Long et al., 2016). The prevalence of NAFLD was 29.2% as determined by CT of which advanced fibrosis was 4%, 5%, 12% and 32% as predicted by the FIB-4, APRI, NFS and AST:ALT, correspondingly (Long et al., 2016). Previous population-based studies have reported that approximately 4% of those living with NAFLD have advanced fibrosis as determined by transient elastography (Wong et al., 2012). Collectively, these data suggest that complex (APRI and NFS), as opposed to simple, composite hepatic fibrosis panels provide a realistic estimate of hepatic fibrosis compared with more criterion methods. Moreover, when using liver biopsy as the reference standard, it has been shown that complex models have greater diagnostic accuracy (Adams et al., 2011; Xiao et al., 2017).

It is pertinent to note that the majority of the proxy fibrosis panels were initially developed for chronic hepatitis C and subsequently modified for NAFLD (Castera et al., 2019; Patel & Sebastiani, 2020). Indeed, the NFS is the only non-proprietary model specifically designed for determining the absence and presence of advanced fibrosis in NAFLD. More

recently, attempts have been made to further improve the diagnostic performance of the NFS and also the FIB-4 with the addition of age-adjusted thresholds for advanced fibrosis (Castera et al., 2019; McPherson et al., 2017). Similar efforts have also been made to improve the accuracy of these composite panels in morbidly obese patients (Meneses et al., 2020).

Somewhat surprisingly, the prevalence of advanced fibrosis as predicted by the non-invasive panels was similar in both participants with presumed NAFLD and those in which NAFLD was predicted to be unlikely (see Table 4.3). This may be a further pitfall related to the initial development of many of the composite models in other liver aetiologies as highlighted previously. Similarly, these predictive algorithms were developed in cohorts with a higher prevalence of advanced fibrosis and not as screening tools (Patel & Sebastiani, 2020). Therefore, unlike the FLI which can be used to identify patients with hepatic steatosis in the primary care or research setting, caution should be applied when utilising composite panels for the prediction of advanced fibrosis in asymptomatic populations or those with a low pre-test probability of hepatic fibrosis (Long et al., 2016). Moreover, clinical practice guidelines advocate that non-invasive tests for fibrosis should always be interpreted by a specialist in liver disease, according to the clinical context, and considering the results of other investigations (EASL-ALEH, 2015). As such, despite the importance of the early detection and staging of fibrosis and its association with liver-related and all-cause mortality (Angulo et al., 2015; Taylor et al., 2020b); data from the current study and others does not support the use of composite scoring panels to predict fibrosis in the absence of additional risk factors.

4.4.3 Prevalence of NAFLD, metabolic risk factors, and corresponding risk of hepatic fibrosis

Comparisons between the cohort with presumed NAFLD and that in which hepatic steatosis was predicted to be absent identified significant differences in participant characteristics consistent with established risk factors for NAFLD (Lim & Bernstein, 2018; Younossi et al., 2016). Notably, the participants with an FLI ≥ 60, were predominantly male, slightly older with both overall clinical and abdominal obesity (demonstrated by a significantly higher BMI, body fat content and enlarged WC); evidently dyslipidaemic, and more hypertensive with high prevalence of MetS diagnosis (up to 60%) and poor cardiorespiratory fitness (lower predicted $\dot{V}O_{2max}$) compared to those individuals with an FLI < 10 ($p < 0.001$ for all parameters respectively, Table 4.2). Likewise, liver enzymes, especially GGT, were significantly higher but typically within the routine normal reference range and against a background of comparable modest alcohol consumption. These prevalent characteristics and established risk factors were subsequently utilised as predictors to determine their relationship with hepatic steatosis and fibrosis through an iterative statistical modelling process.

The measurement model in which the observed variables are regressed on to the latent variables was successful, as indicated by the significant paths from BMI and WC to adiposity; WC, SBP, HDL inverse, TGs and glucose to MetS; and ALT and GGT to liver fat, respectively (Table 4.5, Figure 4.2). The rationale for using these variables as predictors based on the prevalence data according to FLI score above was therefore justified as part of the theory driven approach. Structural equation modelling identified MetS as a significant predictor of liver fat (0.64, 95% CI [0.55, 0.74], $p < 0.001$), an observation consistent with that of others (Kim et al., 2018; Younossi et al., 2011). Moreover, individuals living with NAFLD typically present with multiple components of

MetS (Lim & Bernstein, 2018; Younossi et al., 2016); as reflected in contemporary guidelines (Eslam et al., 2020a, 2020b; Younossi et al., 2023).

The path between liver fat and the NFS was also significant, although negatively loaded (-0.31, 95% CI [0.21, 0.42], $p < 0.001$, Table 4.5, Figure 4.2). Briefly, the NFS is predictive of advanced fibrosis with classifications of low (< -1.455), intermediate (-1.455 to 0.676), and high risk (> 0.676) (Angulo et al., 2007). This inverse relationship may therefore be reflective of the majority of the cohort having an NFS score below the low cut-off (58.63%, *n* = 299/510, Table 4.2). However, the path from adiposity to the NFS was positive. This appears contradictory as one would expect the direction of the relationship from liver fat and adiposity (as indicated by global [BMI] and regional fat distribution [WC]) to the NFS to be of the same direction. It should be noted that BMI is a parameter included with the NFS algorithm (Angulo et al., 2007). Although BMI and the NFS were mediated by the latent variable of adiposity, as opposed to a direct path between the two, confounding cannot be excluded. While not statistically significant, the path from liver fat to the NFS was negative from the initial model (Table 4.5, Figure 4.1). However, this was deemed appropriate as part of the theory driven approach, i.e., the pathological progression of FLD to fibrosis. Moreover, without this direct path, the NFS would not have been indicated by any other parameter within the initial model.

Notwithstanding this, adiposity accounted for a higher proportion of unique variance in the NFS (0.47, 95% CI [0.37, 0.56], $p < 0.001$, Table 4.5, Figure 4.2). Therefore, our model postulated that MetS was a greater predictor of hepatic steatosis, whereas adiposity, as indicated by BMI and WC, was associated with hepatic fibrosis. Although overweight/obesity, as defined by a BMI $\geq$ 25.0 to 30.0 $kg \cdot m^{-2}$ depending on study population (Younossi et al., 2016), has long been associated with NAFLD and disease progression (Younossi et al., 2016, 2023), it is only more recently that WC has been recognised as an increasingly important risk factor (Fracanzani et al., 2017; Golabi et al.,

2020). Specifically, those individuals that present with lean-NAFLD (a diagnosis of NAFLD with a BMI of 18.5-24.9 $kg \cdot m^{-2}$) but are considered to be abdominally obese as indicated by WC (≥ 88 cm and ≥ 102 cm for females and males, respectively), have been shown to have a greater risk of hepatic fibrosis compared to overweight and obese NAFLD patients with a normal or elevated WC (Fracanzani et al., 2017). Moreover, NAFLD patients considered lean by BMI but obese by WC have significantly higher all-cause mortality in comparison to their lean BMI, normal WC counterparts (Golabi et al., 2020). Furthermore, model invariance for sex, smoking status, physical activity, and alcohol consumption was supported, meaning that path loadings did not differ significantly within these groups.

Therefore, our model suggests that BMI and WC in combination, regardless of sex and lifestyle choices, may be used as simple, non-invasive measures to identify those with an increased risk of developing hepatic fibrosis. These observations are consistent with those of others who have similarly advocated the use of these anthropometric measures as clinically important tools to risk stratify patients with NAFLD (Golabi et al., 2020). Practically, the more frequent use of these simple measurements combined with the FLI may increase the identification of NAFLD and those at high risk of developing hepatic fibrosis in the primary care setting where NAFLD in under-recognised or often an incidental finding (Alexander et al., 2018; Armstrong et al., 2012).

Despite these practical recommendations, the current study has several limitations that require consideration. Firstly, the sample was a largely homogenous cross-sectional cohort and therefore not representative of the general population which may have implications for the application of the findings made. A further caveat was that NAFLD and hepatic fibrosis were determined exclusively by non-invasive predictive panels, as opposed to histology or imaging as with previous studies, and therefore it was not possible to determine the actual prevalence or severity of NAFLD or the agreement between these

methods. Similarly, it is possible that confounding may have occurred between predictors and non-invasive panels given that many predictors also functioned as variables within the predictive algorithms. This was unavoidable in the absence of objective determination of NAFLD and fibrosis by biopsy and/or imaging. However, the use of latent variables within the SEM analysis avoided direct paths between observed data that functioned as both predictors of and variables within the composite panels. Instead, these paths were mediated by the latent terms to minimise confounding.

In summary, the prevalence of NAFLD was 34.9% in the current cohort as estimated by the FLI. This figure is comparable to contemporary regional and global estimates. The presence and severity of hepatic fibrosis varied significantly depending on the composite panel and cut-off utilised. For those individuals with a high likelihood of NAFLD (FLI $\geq$ 60), the prevalence of advanced fibrosis was 0%, 0.79%, 1.57%, 45.67%, and 77.17% as determined by the FIB-4, APRI, NFS, AST:ALT, and BARD, respectively. This suggests that the APRI and NFS may provide a realistic estimate of advanced fibrosis aligned with existing data. The SEM analysis identified BMI and WC as simple anthropometric measurements to identify those at higher risk of hepatic fibrosis. These could be integrated into primary care with little inconvenience to health care professionals or patients to help identify those requiring referral for further specialist led investigation.

Chapter 5 The effect of acute piror exercise on postprandial lipaemia following a mixed nutrient metabolic challenge response in apparently healthy, non-obese males

5.1 Introduction

For many individuals, the majority of their waking hours are spent in the postprandial state (Berry et al., 2020; Edinburgh et al., 2017), owing to frequent eating occasions throughout the day (Kant & Graubard, 2015; Wittig et al., 2017). Postprandial lipid, glucose and insulin dysmetabolism are independent risk factors for obesity and associated disease states including T2DM and atherogenic vascular diseases (Berry et al., 2020; Blaak et al., 2006; Kolovou et al., 2011). The conventional practice of obtaining lipid and glucose measurements following an overnight fast to evaluate cardiometabolic risk are therefore not reflective of the repeated metabolic excursions that occur with frequent meals (Kolovou et al., 2011; Nordestgaard et al., 2016).

Accordingly, contemporary guidelines support the use of non-fasting measurements to reflect habitual eating patterns (Kolovou et al., 2019b; National Clinical Guideline Centre (UK), 2014). Postprandial measurements can be acquired as a random sample (Grundy et al., 2019; Kolovou et al., 2019a), or in response to a metabolic (nutritional) challenge; such as the OGTT (Alberti et al., 1985) or OFTT (Kolovou et al., 2011). The OGTT and OFTT may detect metabolic dysfunction not apparent when utilising fasting measurements by highlighting undesirable postprandial responses known to precede chronic disease (Krug et al., 2012; Mihas et al., 2011). Metabolic challenges are therefore applicable not only to the clinical setting for the purpose of diagnosing metabolic disease in patients, but also early detection of metabolic dysfunction in apparently healthy individuals (Mihas et al., 2011).

However, humans rarely consume macronutrients in isolation. Mixed nutrient metabolic challenges have therefore been proposed (Mohanlal & Holman, 2004; Stroeve et al., 2015). Several earlier studies have demonstrated an accentuated lipaemic response to simultaneous fat and carbohydrate ingestion (Cohen & Schall, 1988; Grant et al., 1994; Singleton et al., 1999). Specifically, the addition of fructose or fructose containing carbohydrates to an oral fat load has been shown to augment postprandial TG responses (Cohen & Schall, 1988; Grant et al., 1994; Saito et al., 2015).

Plasma TG concentrations are reflective of the balance between TG appearance (chylomicron-derived TG from dietary intake and hepatically synthesised TG-rich very low density lipoprotein [VLDL-TG]) and disposal (TG uptake primarily into adipose tissue via LPL) (Hodson & Fielding, 2010; Hodson & Frayn, 2011). Fructose ingestion further increases hepatically synthesised TG by DNL whilst also suppressing fatty acid oxidation, resulting in an increase in VLDL-TG production (Chong et al., 2007; Hodson & Frayn, 2011). Furthermore, the insulinaemic response to fructose is lower compared to glucose (Chong et al., 2007). The insulin suppressing effects on NEFA release from adipose tissue and blunting of hepatic VLDL-TG synthesis are therefore reduced (Frayn et al., 1993; Lewis et al., 1995). The up-regulation of LPL activity by insulin and resulting TG storage are also attenuated (Jensen et al., 1989).

The main dietary sources of fructose include soft drinks, juices, cakes and confectionary (Roberts et al., 2018; Sluik et al., 2015), with accumulating evidence in recent years to suggest associations between fructose consumption, obesity, and poor metabolic health (Febbraio & Karin, 2021; Taskinen et al., 2017). Furthermore, guidelines recommending a reduction in free and added sugar intake have also been published (Tedstone et al., 2015). Both physical activity and exercise are advocated to maintain health and reduce obesity (Piercy et al., 2018; Strasser, 2013). Acute exercise provides a potent stimulus that modifies postprandial metabolism (Burns et al., 2015; Edinburgh et al., 2017; Freese et

al., 2014). Although lipaemic responses have typically been studied in response to an oral fat load following exercise (Freese et al., 2014; Maraki & Sidossis, 2013), prior exercise also mitigates postprandial TG responses to fructose when ingested alone (Egli et al., 2013) or concomitantly with fat (Wilburn et al., 2015); although findings are equivocal (Rowe et al., 2016). These contrasting observations may be a consequence of the differing nutritional composition of the metabolic challenges provided. For example, the inclusion of moderate amounts of fat within the test meals was not consistent with guidelines for an OFTT (Kolovou et al., 2011); and therefore may not necessarily be considered as a high-fat challenge. Similarly, the amount of fructose included some metabolic challenges, although consistent with previous studies, was not representative of typical daily intake (SACN, 2015). Instead, fructose is primarily consumed as sucrose (glucose-fructose), with adults consuming 40-50 g per day, in addition to 15-18 g as free fructose and glucose, respectively (SACN, 2015).

The purpose of this study was to first compare the postprandial responses to a mixed nutrient oral metabolic challenge, with the inclusion of isocaloric amounts of fructose or sucrose, the latter representative of habitual consumption, in addition to a recommended oral fat load. Secondly, the study aimed to determine if acute prior exercise could attenuate the postprandial metabolic responses to either meal.

5.2 Methods

5.2.1 Participants

Apparently healthy adult males volunteered for the current study. Participants were excluded if they were inactive (self-reported physical activity of < 150 minutes per week), had a WC ≥ 94 cm, a BMI ≥ 30.0 $kg \cdot m^{-2}$, a previous medical history of CVD, prediabetes or diabetes mellitus, liver disease, gastrointestinal disease, hypertension, lipid lower medication or a current smoker; as determined at the initial screening visit (see Figure

3.1). All study procedures were prior approved by the Faculty of Health Sciences Research Ethics Committee, the University of Hull (REF FHS83 [22/10/2018]) in accordance with the Declaration of Helsinki. All participants provided their written informed consent before any procedures were undertaken. Participant characteristics are presented in Table 5.1 of the Results section.

5.2.2 Study design

The study followed a randomised, single blind, repeated measures cross-over design, and comprised of an initial screening visit and four subsequent visits to undertake each intervention (as detailed in Figure 3.1). Briefly, on two visits participants consumed OFTT-Sucr and on two visits (OFTT-Fruc). Each mixed nutrient metabolic challenge was preceded by rest (OFTT-Sucr and OFTT-Fruc) or prior exercise (OFTT-Sucr-Ex and OFTT-Fruc-Ex). Exercise visits were scheduled between 16:00-19:00 and participants returned to the laboratory the following morning. Participants were also provided with a convenience meal to replace their evening meal and were instructed to consume this before 20:00. All morning visits, including initial screening, were conducted in the fasted state (≥ 12 hours postprandial), no later than 09:30 and separated by ≥ 72 hours.

5.2.3 Outcome measures

The primary outcome measure was the TG tAUC response to OFTT. Secondary outcomes measures included TG iAUC, tAUC responses for glucose, insulin, TC, HDL-c, Apo B, AST, ALT, and GGT. Baseline (fasting) measurements for TGs, glucose and insulin were also analysed.

5.2.4 Statistical analysis

An *a priori* sample size estimation (G*Power, version 3.1.9.6) determined that eight participants were required to achieve 80% power at an alpha level 0.05 based on an effect

size of 0.6 (Freese et al., 2014) and a repeatability of 0.8 (Weiss et al., 2008) for the primary outcome measure of TG tAUC. Normal (Gaussian) distribution of the data was confirmed by exploratory data analysis using visual inspection of frequency histograms, skewness and kurtosis values and the Shapiro-Wilk test ($p > 0.05$). Normally distributed data are reported as mean and (SD, ±), and non-normally distributed data (TG baseline and $\dot{V}O_{2peak}$) as median and inter-quartile range (IQR).

Peak values for HR and RPE (Borg, 1998) obtained during the exercise conditions were compared using paired t-tests and effects sizes calculated using Cohen's *d* (Cohen, 1988). The magnitudes of the effects were interpreted as small (0.2), medium (0.5), and large (0.8), respectively (Cohen, 1988). The tAUC and iAUC for primary and secondary outcome measures were calculated using Microsoft Excel (2016, Microsoft Incorporated, USA) (Narang et al., 2020). A 2 x 2 repeated measures ANOVA was used to determine the main and interaction effects between outcome measures for normally distributed (TG iAUC, glucose tAUC, baseline glucose concentration, TC tAUC, HDL-c tAUC, AST tAUC, ALT tAUC, and GGT tAUC) and transformed data (TG tAUC, and baseline insulin concentrations). Non-normally distributed data that could not be transformed was also included in the analysis (TG baseline, insulin tAUC, and Apo B tAUC). Carbohydrate type (sucrose and fructose) and activity (rest and exercise) were entered as study conditions. Therefore, the differences between outcome measures for each condition and the interaction between conditions could be determined. Where a significant effect was observed, multiple pairwise comparisons with *post hoc* Bonferonni corrections were utilised to locate any differences. Mean differences (MD) with 95% confidence intervals (95% CI), p values and effect size (Partial Eta Squared, ηp^2) are reported. Statistical significance was determined at an alpha level of 0.05 and ηp^2 effects sizes of 0.01, 0.06 and 0.14 were considered small, medium and large, respectively (Cohen, 1988). All statistical procedures were performed using SPSS (Version 26, SPSS

Incorporated, IBM, USA). Figures were created using GraphPad Prism (Version 9.3.1, Graphstats, USA).

5.3 Results

5.3.1 Participant characteristics

Eight of nine male participants completed all study visits (characteristics are summarised in Table 5.1). One male participant attended the screening visit and satisfied the inclusion criteria however discontinued his participant for personal reasons. None of the participants met the criteria for MetS (Alberti et al., 2009) or NAFLD (FLI ≥ 60) (Bedogni et al., 2006).

5.3.2 Supervised exercise interventions

All participants completed the two supervised submaximal HIIE sessions. The mean (SD) power output (W) for the high-intensity and recovery intervals was 192 ± 25 W and 76 ± 10 W, respectively. The peak HR ($b \cdot min^{-1}$) attained during the exercise sessions was 85.6 ± 6.9% of peak HR recorded during CPET; and were not significantly different between the two visits (OFTT-Sucr-Ex: 159 ± 16 $b \cdot min^{-1}$; OFTT-Fruc-Ex: 161 ± 17; MD = -3 $b \cdot min^{-1}$, 95% CI = -8 to 2 $b \cdot min^{-1}$, p = 0.275, Cohen's *d* = 0.42). The peak RPE recorded during exercise was 15 ± 2; and did not differ between exercise sessions (OFTT-Sucr-Ex: 16 ± 2; OFTT-Fruc-Ex: 15 [2]; MD = 1.0, 95% CI = 0.0 to 1.0, p = 0.170, Cohen's *d* = 0.54).

Table 5.1 Participant characteristics

Number of participants	8 male
Age (years)	25 (2)
Mass (kg)	79.2 (6.8)
BMI ($kg \cdot m^{-2}$)	25.1 (1.7)
Waist circumference (cm)	85.4 (4.2)
Waist to hip ratio	0.86 (0.03)
Body fat content (%)	15.3 (1.4)
Blood pressure (mmHg)	127 (8) / 69 (5)
$\dot{V}O_{2peak}$ ($l \cdot min^{-1}$)	3.26 (0.32)
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	40.1 (5.3)#
% predicted normative value†	100.3 (10.7)
$\dot{V}O_{2peak}$ ($ml \cdot kg\text{-}ffm^{-1} \cdot min^{-1}$)	47.1 (7.4)#
Glucose OGTT baseline ($mmol \cdot l^{-1}$)‡	5.10 (0.29)
Glucose OGTT 2 hour ($mmol \cdot l^{-1}$)	3.56 (1.01)
TG ($mmol \cdot l^{-1}$)‡	0.94 (0.35)#
HDL-c ($mmol \cdot l^{-1}$)‡	1.71 (0.41)
LDL-c ($mmol \cdot l^{-1}$)‡	3.19 (0.54)
Total cholesterol ($mmol \cdot l\text{-}^{1}$)‡	5.39 (0.54)
Apo B ($g \cdot l^{-1}$)‡	0.87 (0.19)
AST ($U \cdot l^{-1}$)‡	35 (11)
ALT ($U \cdot l^{-1}$)‡	35 (18)
GGT ($U \cdot l^{-1}$)‡	25 (8)
FLI $\geq$ 60	n = 0

Data are presented as mean and (SD). #Denotes median (IQR). †Predicted normative value calculated according to Wasserman et al. (2004) p.166. ‡Denotes blood samples acquired in the fasted state. ffm; fat free mass.

5.3.3 Primary outcome

5.3.3.1 Total AUC TG responses to OFTT

The TG tAUC responses are shown in Table 5.2. There was a large effect size for a higher TG tAUC response to the two fructose conditions compared to the two sucrose conditions ($\eta p^2 = 0.289$), however, this was not significantly different (MD = 0.08 $mmol \cdot 4hr^{-1} \cdot l^{-1}$, 95% CI = -0.03 to 0.19, p = 0.136). There was no difference in the TG tAUC response between rest and prior evening exercise (MD = 0.03 $mmol \cdot 4hr^{-1} \cdot l^{-1}$, 95% CI = -0.16 to 0.23, p = 0.702, $\eta p^2 = 0.022$). There was no interaction between experimental conditions on TG tAUC (p = 0.807, $\eta p^2 = 0.009$).

5.3.4 Secondary outcomes

The postprandial responses for each of the secondary outcome measures below are shown in Table 5.2.

5.3.4.1 Incremental AUC TG responses to OFTT

The TG iAUC response was significantly higher (1.03 $mmol \cdot 4hr^{-1} \cdot l^{-1}$) in the two fructose conditions compared to the two sucrose conditions (95% CI = 0.18 to 1.88, p = 0.024, $\eta p^2 = 0.542$, Figure 5.1). *Post hoc* analysis showed a significant trend towards a higher TG iAUC response when comparing OFTT-Fruc to OFTT-Sucr (MD = 0.84 $mmol \cdot 4hr^{-1} \cdot l^{-1}$, 95% CI = -0.002 to 1.69, p = 0.051, $\eta p^2 = 0.443$) but not OFTT-Fruc-Ex to OFTT-Sucr-Ex (MD = 1.23 $mmol \cdot 4hr^{-1} \cdot l^{-1}$, 95% CI = -0.38 to 2.83, p = 0.114, $\eta p^2 = 0.317$). There were no differences in TG iAUC response between rest and exercise conditions (0.06 $mmol \cdot 4hr^{-1} \cdot l^{-1}$, 95% CI = -0.97 to 1.09, p = 0.900, $\eta p^2 = 0.002$). There was no interaction effect between interventions on TG iAUC responses (p = 0.651, $\eta p^2 = 0.031$).

5.3.4.2 Baseline TG concentrations

There was no difference in baseline TG concentrations between the two exercise conditions compared to the two rest conditions (MD = 0.08 $mmol \cdot l^{-1}$, 95% CI = -0.301 to 0.468, p = 0.624, $\eta p^2 = 0.036$). However, there was a large effect size ($\eta p^2 = 0.203$) for higher baseline TG concentrations in the two sucrose conditions compared to the two fructose (MD = 0.09 $mmol \cdot l^{-1}$, 95% CI = -0.067 to 0.242, p = 0.224). There was no interaction between experimental conditions on TG baseline concentrations (p = 0.475, $\eta p^2 = 0.075$).

5.3.4.3 Apolipoprotein B tAUC and baseline responses to OFTT.

Average (median and IQR) Apo B responses to OFTT are shown in Figure 5.1. There was large effect size ($\eta p^2 = 0.361$) for a higher Apo B tAUC response to the two sucrose conditions compared to the two fructose conditions (MD = 0.30 $g \cdot 4hr^{-1} \cdot l^{-1}$, 95% CI = -0.06 to 0.65, p = 0.087). There were no effects for evening exercise on Apo B tAUC (MD = 0.04 $g \cdot 4hr^{-1} \cdot l^{-1}$, 95% CI = -0.12 to 0.21, p = 0.563, $\eta p^2 = 0.050$). There was no interaction between interventions on the Apo B tAUC response (p = 0.822, $\eta p^2 = 0.008$).

There was a large effect size ($\eta p^2 = 0.254$) for a higher Apo B baseline concentration to sucrose compared to fructose (MD = 0.06 $g \cdot l^{-1}$, 95% CI = -0.032 to 0.151, p = 0.166). No differences were observed between the two rest conditions compared to the two exercise conditions (MD = 0.03 $g \cdot l^{-1}$, 95% CI = -0.041 to 0.099, p = 0.356, $\eta p^2 = 0.123$). There was no interaction between the interventions on baseline Apo B concentrations (p = 0.616, $\eta p^2 = 0.038$).

5.3.4.4 Glucose and insulin tAUC to OFTT and baseline concentrations

Average (mean [SD] and median [IQR]) tAUC responses to OFTT for glucose and insulin are presented in Figure 5.1, respectively. There was a large effect size ($\eta p^2 = 0.336$) for a

lower glucose tAUC in the two exercise conditions compared to the two rest conditions (MD = 0.66 mmol·4hr^{-1}·l^{-1}, 95% CI = -0.17 to 1.50, p = 0.102). There was no difference in the glucose tAUC response between the fructose and sucrose conditions MD = 0.16 mmol·4hr^{-1}·l^{-1}, 95% CI = -0.62 to 0.95, p = 0.638, ηp^2 = 0.033). There was no interaction between interventions on glucose tAUC responses (p = 0.694, ηp^2 = 0.023).

There was a large effect size (ηp^2 = 0.258) for a lower insulin tAUC response to the two exercise conditions compared to the two rest conditions (MD = 5.40 uIU·4hr^{-1}·ml^{-1}, 95% CI = -2.78 to 13.59, p = 0.162). There were no differences in the insulin tAUC response between the fructose and sucrose conditions (MD = 1.80 uIU·4hr^{-1}·ml^{-1}, 95% CI = -3.18 to 6.78, p = 0.420, ηp^2 = 0.095). There was no statistically significant interaction effect between experimental conditions on insulin tAUC responses (p = 0.426, ηp^2 = 0.092).

There was a large effect size (ηp^2 = 0.268) for lower baseline glucose concentrations in the two exercise conditions compared to the two rest conditions (MD = 0.18 mmol·l^{-1}, 95% CI = -0.09 to 0.45, p = 0.153). There was also a large effect (ηp^2 = 0.268) for lower baseline glucose concentrations prior to sucrose compared to fructose ingestion (MD = -0.12 mmol·l^{-1}, 95% CI = -0.30 to 0.10, p = 0.153). There was no interaction effect between interventions on baseline glucose concentrations (p = 0.580, ηp^2 = 0.046).

There was a large effect size (ηp^2 = 0.324) for lower baseline insulin concentrations in the exercise conditions compared to the two rest conditions (MD = -0.20 uIU·ml^{-1}, 95% CI = -0.46 to 0.06, p = 0.110). There was no difference in the baseline insulin concentrations between fructose and sucrose conditions (MD = 0.03 uIU·ml^{-1}, 95% CI = -0.26 to 0.32, p = 0.826, ηp^2 = 0.007). There was no interaction effect between experimental conditions on baseline insulin concentrations (p = 0.589, ηp^2 = 0.044).

5.3.4.5 TC and HDL tAUC responses to OFTT

There was no effect for the type of carbohydrate included in the OFTT (MD = 0.51 mmol·4hr^{-1}·l^{-1}, 95% CI = -1.86 to 2.88, p = 0.628, ηp^2 = 0.035) or evening exercise (MD = 0.42 mmol·4hr^{-1}·l^{-1}, 95% CI = -1.71 to 0.87, p = 0.465, ηp^2 = 0.079) on the TC tAUC response. However, there was a large interaction effect between the interventions on TC tAUC (p = 0.192, ηp^2 = 0.229).

There were no differences in the HDL tAUC response with fructose included in the OFTT (0.02 mmol·4hr^{-1}·l^{-1}, 95% CI = -0.92 to 0.96, p = 0.960, ηp^2 < 0.001) or following prior evening exercise (0.07 mmol·4hr^{-1}·l^{-1}, 95% CI = -0.34 to 0.48, p = 0.710, ηp^2 = 0.021). However, there was a large interaction between experimental conditions on HDL tAUC (p = 0.122, ηp^2 = 0.307).

5.3.4.6 AST, ALT and GGT tAUC responses to OFTT

There was a large effect size (ηp^2 = 0.206) for a higher AST tAUC response to the two fructose conditions compared to the two sucrose conditions (MD = 18.72 U·4hr^{-1}·l^{-1}, 95% CI = -51.60 to 14.17, p = 0.220). There was no effect for prior evening exercise on the AST tAUC response (MD = 2.53 U·4hr^{-1}·l^{-1}, 95% CI = -24.71 to 29.78, p = 0.832, ηp^2 = 0.007). There was no interaction between experimental conditions on AST tAUC (p = 0.331, ηp^2 = 0.135).

There was no effect for the type of carbohydrate included in the OFTT (MD = 2.66 U·4hr^{-1}·l^{-1}, 95% CI = -21.98 to 27.29, p = 0.806, ηp^2 = 0.009) or exercise (MD = 3.47 U·4hr^{-1}·l^{-1}, 95% CI = -23.32 to 30.26, p = 0.768, ηp^2 = 0.013) on ALT. There was no interaction between interventions on the ALT tAUC response (p = 0.832, ηp^2 = 0.007).

There were no differences with the addition of fructose to the OFTT (MD = 5.25 U·4hr^{-1}·l^{-1}, 95% CI = -8.32 to 18.820, p = 0.391, ηp^2 = 0.107) or following prior evening exercise

(MD = 3.44 U·4hr^{-1}·l^{-1}, 95% CI = -13.23 to 6.36, p = 0.434, ηp^2 = 0.090) on GGT. Finally, there was no interaction between experimental conditions on GGT tAUC (p = 0.934, ηp^2 = 0.001).

Table 5.2 Baseline and postprandial metabolic response to the mixed nutrient metabolic challenge for each study condition.

Variable	OFTT-Sucr	OFTT-Sucr-Ex	OFTT-Fruc	OFTT-Fruc-Ex	Effect size CHO type (ηp2)	p value CHO type	Effect size exercise (ηp2)	p value exercise
TG tAUC (mmol·4hr^{-1}·l^{-1})#	7.44 (4.10)	7.01 (4.18)	8.72 (5.70)	7.37 (4.10)	0.289	0.136	0.022	0.702
TG iAUC (mmol·4hr^{-1}·l^{-1})	2.68 (1.72)	2.43 (1.31)	3.52 (1.68)*	3.66 (2.10)*	0.542	0.024*	0.002	0.900
TG baseline (mmol·l^{-1})#	1.10 (0.58)	1.04 (0.53)	1.11 (0.70)	1.01 (0.32)	0.203	0.224	0.036	0.624
Glucose tAUC (mmol·4hr^{-1}·l^{-1})	19.74 (2.31)	18.83 (1.73)	19.65 (1.70)	19.24 (2.53)	0.023	0.694	0.336	0.102
Glucose baseline (mmol·l^{-1})	5.28 (0.40)	5.08 (0.25)	5.38 (0.43)	5.22 (0.46)	0.268	0.153	0.268	0.153
Insulin tAUC (uIU·4hr^{-1}·ml^{-1})#	30.24 (23.46)	26.08 (18.99)	29.83 (19.12)	25.73 (21.93)	0.095	0.420	0.258	0.162
Insulin baseline (uIU·ml^{-1})#	4.88 (6.31)	4.35 (2.96)	5.63 (2.66)	5.02 (1.77)	0.007	0.826	0.324	0.110
TC tAUC (mmol·4hr-$^{-1}$·l^{-1})	22.23 (1.22)	21.25 (2.87)	20.33 (3.21)	22.14 (3.68)	0.035	0.628	0.465	0.079
HDL tAUC (mmol·4hr^{-1}·l^{-1})	6.68 (1.58)	6.18 (1.60)	6.27 (1.34)	6.63 (1.37)	< 0.001	0.960	0.021	0.710

Data are presented as mean (SD). #Denotes median (IQR). CHO; carbohydrate. Effect size calculated as Partial Eta Squared (ηp^2). *Statistically significant main effect for carbohydrate type.

Table 5.2 continued Baseline and postprandial metabolic responses to the mixed nutrient metabolic challenge for each study condition.

Variable	OFTT-Sucr	OFTT-Sucr-Ex	OFTT-Fruc	OFTT-Fruc-Ex	Effect size CHO type (ηp2)	p value CHO type	Effect size exercise (ηp2)	p value exercise
Apo B tAUC ($g \cdot 4hr^{-1} \cdot l^{-1}$)#	3.81 (0.47)	3.97 (1.18)	3.70 (1.01)	3.83 (0.88)	0.021	0.710	0.361	0.087
Apo B baseline ($g \cdot l^{-1}$)	0.97 (0.24)	0.93 (0.19)	0.90 (0.20)	0.89 (0.17)	0.254	0.166	0.123	0.356
AST tAUC ($U \cdot 4hr^{-1} \cdot l^{-1}$)	127 (28)	137 (49)	153 (66)	148 (46)	0.206	0.220	0.007	0.832
ALT tAUC ($U \cdot 4hr^{-1} \cdot l^{-1}$)	138 (67)	139 (55)	133 (55)	138 (49)	0.009	0.806	0.013	0.768
GGT tAUC ($U.4hr^{-1} \cdot l^{-1}$)	84 (19)	88 (31)	79 (34)	82 (26)	0.107	0.391	0.090	0.434

Data are presented as mean (SD). #Denotes median (IQR). CHO; carbohydrate. Effect size calculated as Partial Eta Squared (ηp^2). *Statistically significant main effect for carbohydrate type.

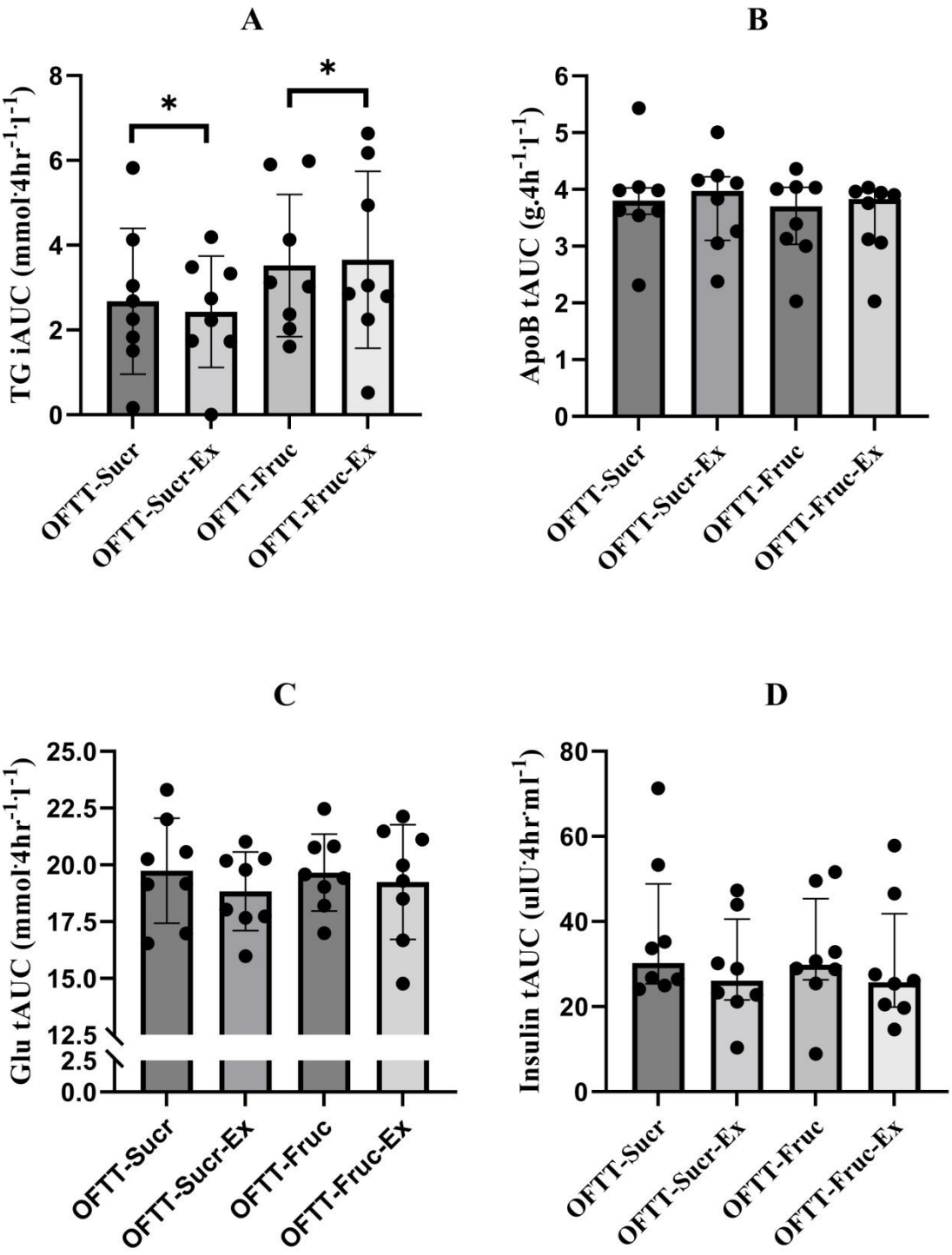

Figure 5.1 Postprandial triglyceride (TG), apolipoprotein B (Apo B), glucose (Glu) and insulin responses to each study condition. Panel A: mean (SD) TG iAUC; panel B: median (IQR) Apo B tAUC; panel C: mean (SD) Glu tAUC; panel D: median (IQR) insulin tAUC responses to the respective study conditions. Each data point represents the individual response to each condition, respectively. *Denotes a statistically significant (p = 0.024) main effect for carbohydrate type.

5.4 Discussion

The present study investigated the postprandial metabolic responses to a mixed nutrient oral challenge (with the inclusion of fructose and an equivalent fat load combined with sucrose), following prior evening acute submaximal HIIE or no physical activity condition. The primary findings were that fructose combined with OFTT significantly increased the TG iAUC response compared to an iso-caloric sucrose and fat solution (Figure 5.1). A large effect size was also observed for an increase in TG tAUC with fructose consumption. Acute exercise did not attenuate the postprandial TG response to either mixed nutrient meal. However, secondary outcome measures demonstrated large effect sizes for lower fasting and postprandial glucose and insulin measurements following prior evening exercise.

5.4.1 Fructose versus sucrose consumption and postprandial metabolic responses

Although it is well established that the addition of fructose or fructose containing sugars (i.e. sucrose) to an oral fat load exacerbates the postprandial TG response (Cohen & Schall, 1988; Grant et al., 1994; Saito et al., 2013; Singleton et al., 1999), few studies have directly compared the postprandial responses to fructose *versus* sucrose co-ingestion with fat (Cohen & Schall, 1988; Gallagher et al., 2016; Saito et al., 2015). Our data show a ~ 40% higher TG iAUC response to fructose compared to sucrose inclusion within an OFTT (approximately 1 $mmol \cdot 4hr^{-1} \cdot l^{-1}$, Table 5.2) within non-obese, physically active participants with low metabolic risk.

Notably, the increase in TG iAUC with fructose consumption was observed despite comparable insulin, glucose, and Apo B responses to that of sucrose inclusion within the OFTT (Figure 5.1). Previous studies have proposed that a lower insulinaemic response

observed with fructose ingestion has the potential to elevate TG concentrations via several mechanisms, including lower LPL activation and subsequent TG clearance into adipose tissue (Chong et al., 2007; Sadur & Eckel, 1982); reduced suppression of adipose tissue lipolysis and continued NEFA availability (Frayn et al., 1993; Jensen et al., 1989); and reduced inhibition of hepatic VLDL-TG production (Lewis et al., 1995). Our data therefore suggest that a mechanism independent of, or in addition to insulin, may be responsible for the pronounced TG excursions with fructose consumption.

Hepatic DNL is a further regulator of TG metabolism for which fructose acts as both a precursor and stimulator (Chong et al., 2007; Hengist et al., 2019). Although the direct conversion of fructose into *de novo* fatty acids and the subsequent contribution to postprandial lipaemia is small (Chong et al., 2007), the stimulation of DNL by fructose could increase the conversion of non-lipid precursors such a glucose, fructose and lactate into fatty acids and glycerol (Hengist et al., 2019; Watkins et al., 2020). The acute increase in DNL in the presence of fructose is thought to be due to the rapid and largely unregulated metabolism of fructose within the liver (Hengist et al., 2019; Watkins et al., 2020). This results in the accumulation of triose phosphate, which could be converted into TGs, lactate, and/or glucose and glycogen (Hengist et al., 2019; Watkins et al., 2020).

The DNL pathway is also stimulated by insulin and transitions hepatic metabolism from oxidation to esterification (Hodson & Frayn, 2011; Hodson & Gunn, 2019b). Insulin up-regulates the transcription factor SREBP-1c and in turn enhances the transcription of genes needed for fatty acid and TG synthesis (Brown & Goldstein, 2008; Low et al., 2018). The activation of SREBP-1c can also be achieved with fructose feeding independent of insulin (Hengist et al., 2019). Low and colleagues (2018) have postulated that fructose may stimulate the transcription of SREBP-1c and ChREBP via PPAR-γ coactivator 1β, which is a transcriptional coactivator for SREBP-1c (Low et al., 2018; Nagai et al., 2009; Pinnick & Hodson, 2019). Furthermore, fructose-1, 6-bisphosphate, an intermediate in

the pentose pathway, can combine with glyceraldehydes to synthesise xylulose-5-phosphate (Bonsignore et al., 1962), an up-regulator of protein phosphatase 2A (Kabashima et al., 2003) and can subsequently activate ChREBP (Dentin et al., 2006; Low et al., 2018). Therefore, it is plausible that fructose and insulin had synergistic effects on hepatic DNL and augmented the TG iAUC response to OFTT-Fruc (Low et al., 2018).

Contemporary data have also highlighted the increasingly recognised role of the intestine in fructose metabolism (Hoffman et al., 2019; Jang et al., 2018). Until recently, orally ingested fructose was thought to be primarily metabolised within the liver (Jang et al., 2018). An evolving concept is that enterocytes may contribute to fructose induced lipaemia via intestinal DNL and/or the metabolism of non-lipid precursors for hepatic DNL (Steenson et al., 2020; Theytaz et al., 2014). Although further investigation is required, these novel mechanisms may further explain the increased TG iAUC observed with OFTT-Fruc compared to OFTT-Sucr in the absence of significant differences in insulinaemic and glycaemic responses.

Similarly, no statistically significant differences were observed in the Apo B responses to either metabolic challenge. Apo B isoforms 100 and 48 are indicative of hepatic VLDL-TG and intestinal chylomicron TG metabolism, respectively (Frayn and Evans, 2019). An increase in either subfraction therefore has the potential to increase net Apo B appearance. Given the significant increases in TG iAUC, it may be assumed that Apo B would also increase. However, in the absence of measuring these isoforms directly, it is challenging to determine their rate of appearance or clearance and subsequent impact on Apo B. Previous data from our laboratory has demonstrated that Apo B concentrations are less susceptible to acute changes compared to TG concentrations when utilising the same OFTT (without sucrose or fructose) in apparently healthy male participants (O'Doherty et al., 2018).

Finally, although a large effect size was observed, the TG tAUC was not significantly different between fructose and sucrose inclusion within the OFTT despite significant differences in TG iAUC. Briefly, the tAUC quantifies the overall exposure to the outcome of interest. However, it may be limited by variations in baseline data despite efforts to replicate each study condition (Narang et al., 2020). Indeed, a large effect size was observed for higher fasting TG concentrations prior to sucrose and fat compared to fructose and fat ingestion which may have contributed to the observed non-significant differences in the tAUC response. To overcome this and/or when the response to a stimulus is of primary interest, the iAUC relative to a nominal value (typically baseline) may be a more appropriate metric (Carstensen et al., 2003; Narang et al., 2020). Despite this, both measures are commonly utilised in the reporting of postprandial metabolic responses (Lee et al., 2020). The selection of either the tAUC or iAUC to quantify primary outcomes should therefore be an important consideration in the study design process.

5.4.2 Exercise and postprandial metabolic responses to fructose and sucrose

Although acute prior evening exercise did not modify the TG response, large effects were observed for a reduction in fasting and postprandial glucose and insulin concentrations, respectively. Acute exercise increases muscle glucose uptake by both insulin-dependent and independent mechanisms (Edinburgh et al., 2017). Muscle contractions stimulate GLUT4 translocation to the cell membrane and facilitate glucose transport into the muscle (Lund et al., 1995). Insulin-stimulated glucose transport by GLUT4 is also elevated for at least 16 hours post-exercise (Edinburgh et al., 2017; Mikines et al., 1988); a similar window between exercise and OFTT ingestion to that of the current study. However, the mechanisms responsible for contraction and insulin-induced glucose uptake facilitated by GLUT4 are thought to be distinct (Lund et al., 1995; Park et al., 2014). Briefly, although calcium (Ca^{2+}) is needed for GLUT4 translocation, it is the use of Ca^{2+} secondary messengers, specifically cyclic ADP-ribose (cADPR) during muscle contraction and *D*-

myo-inositol 1,4,5-trisphosphate (IP_3) by insulin that differentiate between the two stimuli. This explains the basis for improved glucose uptake post exercise in insulin resistant individuals (Park et al., 2014). These beneficial effects are widely acknowledged, with exercise regarded as an effective non-pharmacological strategy for improving postprandial glucose control (Edinburgh et al., 2017).

With reference to the postprandial TG responses, the weight of current evidence suggests that exercise performed 8-24 hours prior to the ingestion of a high-fat meal reduces the magnitude of postprandial TG excursions (Burns et al., 2015; Freese et al., 2014; Maraki & Sidossis, 2013). Moreover, HIIE appears to further reduce TG responses compared to moderate continuous exercise (Burns et al., 2015; Freese et al., 2014). Our observation that acute exercise failed to attenuate the TG response to either metabolic challenge is therefore contradictory to the majority of the existing prior exercise literature. However, few studies have examined the potential benefits of acute exercise on the postprandial responses to concomitant oral fat and fructose ingestion (Macedo et al., 2019; Rowe et al., 2016; Wilburn et al., 2015).

Similar to our study, Rowe and colleagues (2016) reported no differences in the postprandial TG response to a mixed meal containing either glucose or fructose following exercise compared to rest. In this study, sedentary women completed four trials and the exercise intervention expended 500 Kcal while walking at 70% $\dot{V}O_{2max}$. The effect of the prior acute exercise was quantified by the TG iAUC method over a 6-hour postprandial period. There was no significant difference in the TG iAUC between the four trials. In contrast, Macedo and associates (2019) observed a ~ 30% reduction in the TG tAUC to oral fat and fructose ingestion with prior exercise in 12 young, lean but sedentary men. A commonality of these studies was that exercise was performed in the late afternoon or early evening with participants returning to the laboratory the following morning after an overnight fast (Macedo et al., 2019; Rowe et al., 2016). The duration of the exercise

training interventions, approximately 45 minutes, were also similar (Macedo et al., 2019; Rowe et al., 2016). However, there were notable differences in the methods of exercise prescription. For example, Rowe and co-investigators (2016) terminated exercise sessions when participants had expended 500 Kcal by walking at 70% $\dot{V}O_{2max}$. Total exercise time was 83.9 ± 12.6 minutes and 84.0 ± 11.9 minutes for the exercise-glucose and exercise-fructose conditions, respectively (Rowe et al., 2016). Rather than an energy expenditure target, Macedo and colleagues (2019) required volunteers to walk for 45 minutes at 60% $\dot{V}O_{2max}$. In our study, submaximal HIIE was prescribed according to exercise intensity domains as identified by expired ventilatory gas analysis during a CPET at each participants screening visit and totalled 50 minutes including warm-up and cool-down (Figure 3.2) (O'Doherty et al., 2017; Özyener et al., 2001). This method of exercise prescription, known as the threshold-based approach, has distinct advantages compared to traditional models of programming exercise at percentages of $\dot{V}O_{2max}$ (Mezzani et al., 2013; Wolpern et al., 2015). Exercise anchored to the well-established and reproducible thresholds that identify the transition between exercise intensity domains means that the metabolic demands and therefore relative exercise intensity should have been similar for each participant. Subsequently, our approach represents an individualised and robust method to minimise inter- and intra-individual responses to exercise interventions (Wolpern et al., 2015). It is therefore plausible that the exercise training sessions prescribed by the previously cited studies (Macedo et al., 2019; Rowe et al., 2016) may have produced disparate metabolic responses and hence the equivocal findings.

Post-exercise energy provision is a further methodological consideration that may have attenuated the postprandial metabolic response (Freese et al., 2014; Taylor et al., 2018). In our study, participants consumed a convenience meal unsupervised the evening prior to returning to the laboratory the following morning. This was replicated across all experimental study visits. The rationale for this dietary standardisation was twofold.

Firstly, it has been demonstrated that the effects of an evening meal persist overnight and can modulate postprandial metabolic responses the following morning (Robertson et al., 2002). Secondly, in a free-living environment, individuals would typically consume an evening meal and likely replenish the energy expended during exercise. Therefore, providing an evening meal maintained the ecological validity of the study. Similar to our investigation, Wilburn and colleagues (2015) examined the postprandial responses to simultaneous fat and fructose ingestion following acute exercise, however, with and without post-exercise energy replacement. The authors reported no significant differences in the postprandial TG response between exercise conditions with and without energy compensation, although plasma TGs were further reduced by approximately a third when an energy deficit was allowed to occur (16.5% and 24.4%, respectively) (Wilburn et al., 2015). Others have also presented inconsistent findings when investigating postprandial responses following acute exercise with and without energy replacement (Freese et al., 2011; Harrison et al., 2009). Miyashita et al., (2020) demonstrated the influence of acute exhaustive exercise on postprandial lipid metabolism in eight recreationally active young men. This involved prolonged exercise without carbohydrate replacement and prolonged exercise with carbohydrate replacement to restore energy balance. The effect on postprandial lipaemia was shown to be largely dependent on the associated carbohydrate and energy deficit induced by the acute exercise intervention.

The mean energy intake provided by the evening meals in our study was 552 ± 37 Kcal. However, exercise energy expenditure was not determined as calculations for indirect calorimetry are inaccurate at exercise intensities above AT (Jeukendrup & Wallis, 2005). It is therefore unclear the extent to which this energy displacement was replenished following exercise and the subsequent impact on the postprandial metabolic response. Further studies are needed to delineate not only the effect of energy replacement *per se*,

but also the macronutrient composition and the proximity of subsequent meals to both exercise and the nutritional metabolic challenge itself.

5.4.3 Strengths and limitations

As highlighted above, the current study has several strengths to its methodological design, including the use of robust methods for exercise prescription; standardisation of the evening meal prior to study visits the following morning; the use of iso-caloric mixed nutrient metabolic challenges in which the fat content adhered to recommendations advocated for an OFTT, and the carbohydrate fraction included was comparable to that of habitual intake and previous literature.

A noted caveat of our investigation was the absence of cannulation. Not only would this have minimised the number of venepunctures, but it would have also allowed for more frequent blood sampling and further improved data clarity; including capturing the peaks and troughs in metabolite concentrations and ensuring that the tAUC and iAUC better summarised these responses.

5.4.4 Summary and conclusions

In summary, fructose and oral fat ingestion increased the postprandial TG iAUC response compared to an energy matched sucrose and fat solution. Acute submaximal HIIE did not attenuate the postprandial TG response to either meal. Our data support the need for further studies to elucidate the mechanisms underlying the lipogenic effects of fructose consumption. Moreover, it would be interesting to replicate the current study within a group at higher risk of, or with known, liver-related, metabolic disorders.

Chapter 6 The effects of acute interval exercise on postprandial lipaemia in inactive overweight and centrally obese men at higher risk of NAFLD

6.1 Introduction

Habitual eating behaviours have become increasingly diverse with several meals and snacks consumed throughout the day (Kant & Graubard, 2015; Wittig et al., 2017). With the exception of the hours before waking, most individuals spend the majority of their day in the postprandial state (Berry et al., 2020; Edinburgh et al., 2017). Impaired lipid and glucose handling following a mixed-nutrient meal are associated with poor health outcomes, including obesity and cardiometabolic disease (Berry et al., 2020; Blaak et al., 2006; Pirillo et al., 2014). The primary nutritional factors affecting postprandial excursions include the amount and type of fat and carbohydrate that is ingested (Watkins et al., 2020).

Consuming meals away from the home has also become more frequent (Guthrie et al., 2002; Saksena et al., 2018), with these tending to be energy dense (Guthrie et al., 2002; Lachat et al., 2012). Excess calories are typically delivered through the consumption of high-fat foods and sugar-sweetened beverages (Roberts et al., 2018; Sluik et al., 2015). Fructose and fructose containing sugars are common sweeteners used to increase the palatability of these products. In Europe, the main sources of fructose include soft drinks, fruit juices and confectionary (Roberts et al., 2018; Sluik et al., 2015); with approximately two-thirds of fructose consumed in the form of sucrose and the remainder as free fructose (Hengist et al., 2019; Sluik et al., 2015).

Unlike other monosaccharides, fructose is primarily metabolised within the liver (Tappy & Lê, 2010) and increases TG concentrations compared to glucose (Chong et al., 2007).

Fructose consumption increases hepatically synthesised TG by DNL and suppresses fatty acid oxidation, resulting in greater VLDL-TG production and plasma TG concentrations (Chong et al., 2007; Hodson & Frayn, 2011). In addition, the lower insulinaemic response to fructose limits the insulin stimulated activation of LPL and reduces the clearance of TGs into adipose tissue (Sadur & Eckel, 1982). The insulin-mediated suppression of NEFA release from adipose tissue and blunting of hepatic VLDL-TG synthesis are also reduced (Frayn et al., 1993; Jensen et al., 1989; Lewis et al., 1995).

Unsurprisingly, the increase in fructose consumption over the previous four decades has been associated with obesity, CVD, T2DM, and NAFLD (Tappy & Lê, 2010; Taskinen et al., 2017). Meta-analysis of short-term intervention studies (≥ 7 days) has shown that dietary supplementation with fructose increases postprandial TG concentrations in overweight and obese but not in otherwise healthy individuals (Wang et al., 2014; Hengist et al., 2019). The underlying mechanisms appear to include increases in visceral adipose tissue volume and hepatic DNL, and alterations in lipid metabolism (Stanhope et al., 2008). This suggests that fructose ingestion may only negatively affect metabolic health in a status of positive energy balance and/or when relatively sedentary (Gonzalez & Betts, 2018; Hengist et al., 2019; Tappy & Rosset, 2019).

The early meta-analysis of Freese et al. (2014) concluded that prior acute exercise reduces postprandial lipaemia. A total of 121 acute exercise effects were found from 76 studies for the total TG response and 70 exercise effects from 44 studies for the iAUC TG response. Notably, the weighted mean effect was highly significant, with a moderate magnitude for both the total TG response (Cohen's $d = -0.60$) and iAUC response (Cohen's $d = -0.59$). The magnitude of effect was influenced by sex, type of exercise, and energy deficit following exercise. HIIE induced a larger reduction in the iAUC response ($d = -1.49$) than acute aerobic exercise ($d = -0.58$), and participants maintaining an

energy deficit following exercise exhibited a greater reduction in the iAUC response (d = −0.67) compared with participants in energy balance (d = −0.28).

Pearson et al. (2022) subsequently systematically reviewed and meta-analysed the results from 279 acute exercise intervention effects retrieved from 165 studies for the total TG response; and 142 effects from 87 studies for the iAUC TG response. These investigators incorporated a moderator analysis to examine potential mediating variables. There was a significant moderate effect of exercise on the total TG response (Cohen's d = −0.47). Moderator analysis revealed exercise energy expenditure significantly moderated the effect of prior exercise on the total TG response. Consistent with earlier findings, exercise modality and cardiorespiratory exercise type (e.g., continuous, interval, concurrent, or combined), and timing of exercise prior to meal administration significantly affected the total TG response. Additionally, acute exercise had a moderate effect on the iAUC TG response (Cohen's d = −0.40). However, only three of the studies identified examined the effects of prior exercise on TG responses to a mixed meal with the inclusion of fructose (Macedo et al., 2019; Rowe et al., 2016; Wilburn et al., 2015). The authors concluded that their analysis revealed the literature was more homogenous than previously reported and the attenuation of postprandial TGs appeared largely dependent on exercise energy expenditure and the timing of exercise.

Acute exercise sessions therefore present a strategy to increase energy turnover/deficit and may attenuate the adverse metabolic effects of fructose consumption (Macedo et al., 2019; Rowe et al., 2016; Wilburn et al., 2015). However, fructose is rarely consumed in isolation. Indeed, the addition of fructose and fructose containing sugars (sucrose) to an oral fat load exacerbates the postprandial TG response in apparently healthy individuals (Cohen & Schall, 1988; Grant et al., 1994; Saito et al., 2015). However, as yet, no study has documented the effects of acute exercise on the postprandial responses to fructose

compared with sucrose inclusion within a mixed nutrient metabolic challenge in inactive, overweight and centrally obese individuals.

The aim of this current study was two-fold; first, to compare the postprandial metabolic responses to fructose versus sucrose inclusion within an OFTT in inactive, overweight, and centrally obese adult males, and second, to determine if prior evening exercise could attenuate the postprandial metabolic excursions following oral fat and fructose or oral fat and sucrose ingestion within this cohort.

6.2 Methods

6.2.1 Participants

Inactive, overweight, and obese adult males (self-reported physical activity < 150 minutes per week, BMI ≥ 25.0 $kg \cdot m^{-2}$, and WC ≥ 94 cm) were recruited. Participants presenting with additional MetS criteria, including: upper or above normal blood pressure (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg, or existing antihypertensive drug treatment); elevated fasting TGs (≥ 1.7 $mmol \cdot l^{-1}$ or existing lipid lowering therapy); reduced HDL-c (< 1.0 $mmol \cdot l^{-1}$ or drug treatment); and/or IFG (5.6-6.9 $mmol \cdot l^{-1}$) or impaired glucose tolerance (7.8-11.0 $mmol \cdot l^{-1}$) were eligible for the study (Alberti et al., 2009; American Diabetes Association, 2014). Participants presenting with severe untreated arterial hypertension at rest (> 200 mmHg systolic and/or > 120 mmHg diastolic (Taylor et al., 2015) or with a previous medical history of diabetes mellitus, liver disease, gastrointestinal disease, or a current smoker were excluded from the study. All study procedures were prior approved by the Faculty of Health Sciences Research Ethics Committee, the University of Hull (REF FHS161 [16/07/2019]) in accordance with the Declaration of Helsinki. All participants provided their written informed consent before any procedures were undertaken. A schematic of participant recruitment and

characteristics are presented in Figure 6.1 and Table 6.1 of the Results section, respectively.

6.2.2 Study design

The study followed a randomised, single blind, repeated measures design, and comprised of an initial screening visit and four subsequent visits to undertake each intervention (see Figure 3.1). Briefly, on two visits participants consumed OFTT-Sucr and on two visits OFTT-Fruc. Each mixed nutrient metabolic challenge was preceded by rest (OFTT-Sucr and OFTT-Fruc) or prior exercise (OFTT-Sucr-Ex and OFTT-Fruc-Ex). Exercise visits were scheduled between 16:00-19:00 and participants returned to the laboratory the following morning. Participants were also provided with a convenience meal to replace their evening meal and were instructed to consume this before 20:00. All morning visits, including initial screening, were conducted in the fasted state (≥ 12 hours postprandial), no later than 09:30 and separated by ≥ 72 hours.

Outcome measures

The primary outcome measure was the TG (tAUC) response to OFTT. Secondary outcomes measures included TG iAUC, tAUC responses for glucose, insulin, TC, HDL-c, AST, ALT, and GGT. Baseline (fasting) measurements for TGs, glucose and insulin were also analysed.

6.2.3 Statistical analysis

An *a priori* sample size estimation (G*Power, version 3.1.9.6) was conducted as detailed by previous work from our laboratory (O'Doherty et al., 2017). It was anticipated that the repeatability of the primary outcome measure, TG tAUC, would be high (0.8) (Weiss et al., 2008). Utilising a more conservative estimate of 0.7, an effect size of 0.7 (Freese et al., 2014), an alpha of level 0.05 and 80% power, it was estimated that a sample size of

10 participants was required. Normal (Gaussian) distribution of the data was confirmed by exploratory data analysis using visual inspection of frequency histograms, skewness and kurtosis values and the Shapiro-Wilk test ($p > 0.05$). Normally distributed data are reported as mean and (SD, ±), and non-normally distributed data as median and (IQR).

Blood samples were acquired on 63 out of a possible 70 occasions: with 7 data points (10%) missing due to a sample not being drawn successfully within two attempts. This can be common in time series data, as the probability of error is increased when large numbers of samples are collected (Narang et al., 2020). The missing data mechanism was considered to be *missing completely at random* (MCAR) as missing values were unrelated to the observed data (Little & Rubin, 2002). The MCAR mechanism was further supported as the assumption of Little's MCAR test (Little, 1988) was upheld (Chi-Squared [χ^2] = 3.06, degrees of freedom [*df*] = 63, p = 1.00). Under MCAR, the 'missingness' is considered totally random and therefore participants without missing data are a random subsample of the original sample; meaning analysis using data from completed cases only is valid (Li & Stuart, 2019). However, complete case analysis reduces statistical power as the observed data of those with missing values is not preserved. Imputation methods, including mean imputation using observed values for a given condition, are a strategy for handling data considered MCAR (Li & Stuart, 2019; Little & Rubin, 2002). Missing values were therefore imputed using this method.

Peak values for HR and RPE (Borg, 1998) obtained during the exercise conditions were compared using paired t-tests. Effect sizes were calculated using Cohen's *d* (Cohen, 1988). The magnitudes of the effects were interpreted as small (0.2), medium (0.5), and large (0.8), respectively (Cohen, 1988). Participant characteristics of individuals completing all study visits and those who completed the initial screening visit only are presented in Table 6.1. Normally and non-distributed variables were compared using independent t-tests and

the Mann-Whitney-U test, respectively. Fisher's exact test was used to compare the proportion of participants of with an FLI 30-59, FLI ≥ 60 and MetS, correspondingly. Participant characteristics from the current study and those from Chapter 4 are also compared in Table 6.2. Normally distributed and transformed variables (BMI, WC, body fat content and AST) were analysed using independent t-tests. Non-normally distributed variables and those that could not be transformed (WHR) were compared using the Mann-Whitney-U test. The Chi-square test was utilised to compare the proportion (%) of participants with MetS.

The tAUC and iAUC for primary and secondary outcome measures were calculated using the TSRA (Narang et al., 2020). A 2 x 2 repeated measures ANOVA was used to determine the main and interaction effects between outcome measures for normally distributed (TG tAUC, TG iAUC, fasting TG concentrations, glucose tAUC, fasting glucose concentrations and HDL-c tAUC) and transformed data (insulin tAUC, fasting insulin concentrations, TC tAUC, AST tAUC and ALT tAUC). Non-normally distributed data that could not be transformed was also included in the analysis (GGT tAUC). Carbohydrate type (sucrose and fructose) and activity (no exercise and exercise) were entered as study conditions. Therefore, the differences between outcome measures for each condition and the interaction between conditions could be determined. Where a significant effect was observed, multiple pairwise comparisons with *post hoc* Bonferonni corrections were utilised to locate any differences. Mean differences (MD) with 95% CI, p values and ηp^2 are reported. Statistical significance was determined at an alpha level of 0.05 and ηp^2 effects sizes of 0.01, 0.06 and 0.14 were considered small, medium and large, respectively (Cohen, 1988). All statistical procedures were performed using SPSS (Version 26, SPSS Incorporated, IBM, USA). Figures were created using GraphPad Prism (Version 9.3.1, Graphstats, USA).

6.3 Results

6.3.1 Study flow and participant characteristics

A schematic representation of participant recruitment is shown in Figure 6.1. Five of 11 male participants completed all study visits (participant characteristics, Table 6.1). Due to the sample size achieved, the study design lacked sufficient statistical power to detect significant differences in TG tAUC as determined within the *a priori* sample size estimation. Data should therefore be interpreted with caution. Characteristics of participants recruited to the current study and Chapter 4, from which the inclusion/exclusion criteria were informed, are presented in Table 6.2.

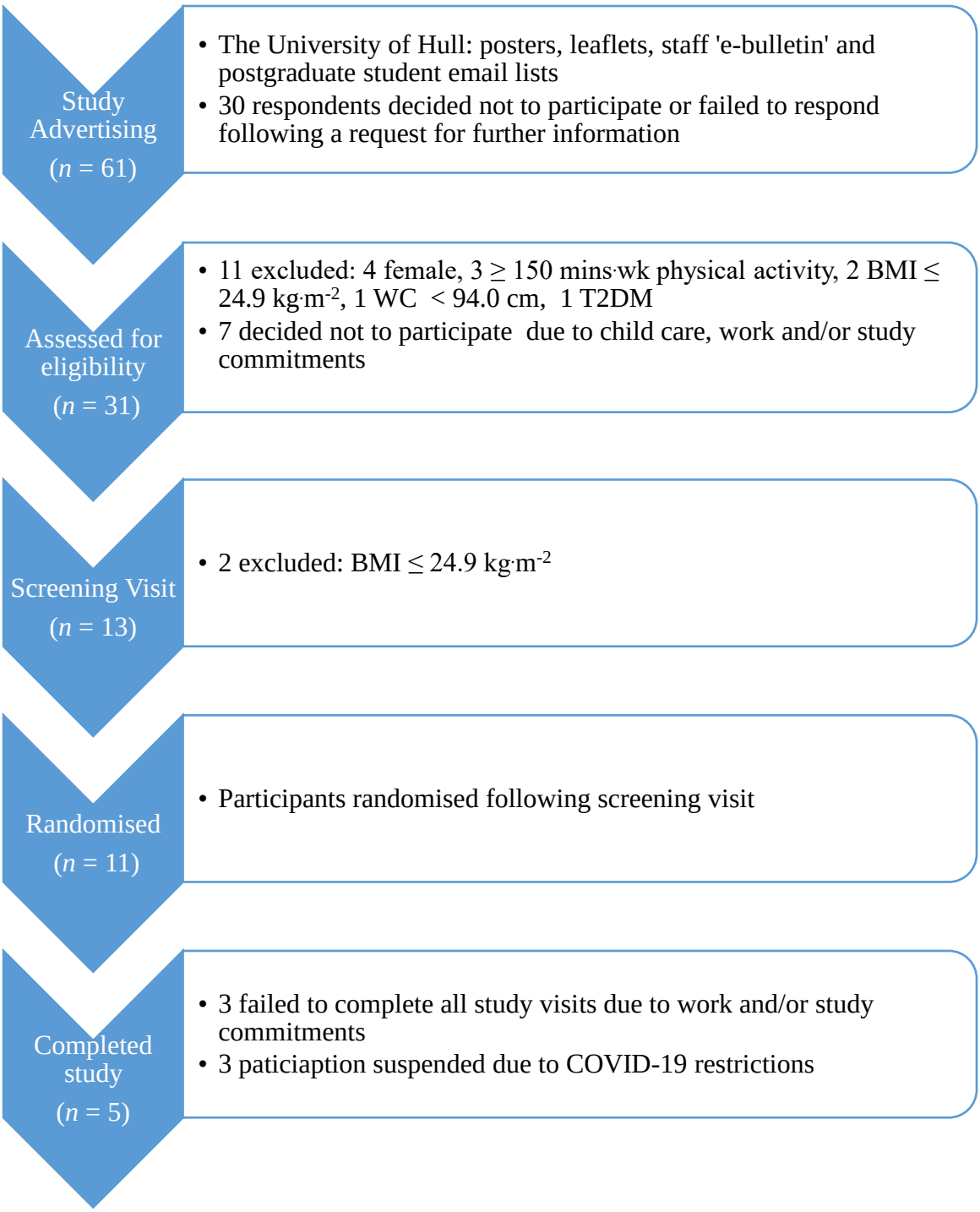

Figure 6.1 Schematic representation of participant recruitment.

Table 6.1 Participant characteristics of those individuals completing all study visits and screening visit only.

Variable	Completed	Non-completed
Number of participants	5	6
Age (years)	54 (20)#	43 (21)#
Mass (kg)	104.1 (20.1)	101.4 (19.3)
BMI ($kg \cdot m^{-2}$)	32.7 (4.2)	32.3 (4.8)
Waist circumference (cm)	113.9 (13.3)	109.1 (12.9)
Waist to hip ratio	1.02 (0.06)	1.00 (0.06)
Body fat content (%)	34.3 (5.0)	32.4 (5.9)
Systolic blood pressure (mmHg)	142 (21	128 (14)
Diastolic blood pressure	81 (7)	83 (10)
$\dot{V}O_2$peak ($l \cdot min^{-1}$)	2.34 (0.45)#	2.44 (0.33)#
$\dot{V}O_2$peak (ml.kg-1.min-1)	23.9 (4.3)	25.3 (4.3)
% predicted normative value¥	92.1 (12.6)	85.3 (7.3)
$\dot{V}O_2$peak ($ml \cdot kg\text{-}ffm^{-1} \cdot min^{-1}$)	36.1 (4.1)	36.9 (4.8)
Glucose baseline ($mmol \cdot l^{-1}$)†	6.01 (0.82)	6.13 (0.39)
Glucose OGTT 2 hour ($mmol \cdot l^{-1}$)	5.40 (1.86)	5.54 (2.1)
TG ($mmol \cdot l^{-1}$)†	1.14 (0.38)	2.45 (1.7)
HDL-c ($mmol \cdot l^{-1}$)†	1.33 (0.10)	1.34 (0.19)
LDL-c ($mmol \cdot l^{-1}$)†	3.29 (0.53)	4.03 (0.92)
Total cholesterol ($mmol \cdot l^{-1}$)†	5.14 (0.52)*	6.48 (1.22)*
AST ($U \cdot l^{-1}$)†	23 (4)*	31 (7)*
ALT ($U \cdot l^{-1}$)†	26 (13)#	55 (40)#
GGT ($U \cdot l^{-1}$)†	20 (29)#	37 (32)#
FLI 30-59 (*n*, %)	1 (20%)	1 (16.7%)
FLI ≥ 60 (*n*, %)	3 (60%)	5 (83.3%)
MetS (*n*, %)‡	3 (60%)	5 (83.3%)

Data are presented as mean and (SD). #Median (IQR). †Blood samples acquired in the fasted state. ¥Predicted normative value calculated according to Wasserman et al. (2004) p.166. ‡Participants presenting with ≥ 3 risk factors indicative of the metabolic syndrome (MetS) (Alberti et al., 2009). *Independent t-test, $p < 0.05$. ^Mann-Whitney-U test, $p < 0.05$. ∨Fisher's exact test, $p < 0.05$.

Table 6.2 Comparison of participant characteristics of individuals completing the screening visit and individuals with an FLI ≥ 60 from the Nuffield Health Screening Study (Thesis Chapter 4).

Variable	Screening visit	FLI ≥ 60 (high-risk FLI cohort within Chapter 4)
Number of participants (female)	11 (0)	127 (6)
Age (years)	45 (13)	49 (7)
Mass (kg)	102.6 (18.7)	98.1 (14.4)
BMI ($kg \cdot m^{-2}$)	32.5 (4.4)	31.0 (3.6)
WC (cm)	111.3 (12.7)	105.4 (10.1)
Waist to hip ratio	1.02 (0.10)#	0.94 (0.06)#^
Body fat content (%)	33.2 (5.3)*	29.8 (5.9)*
Systolic blood pressure (mmHg)	135 (18)	129 (13)
Diastolic blood pressure (mmHg)	82 (9)	85 (8)
$\dot{V}O_{2peak}$ ($l \cdot min^{-1}$)	2.39 (0.28)#	3.01 (0.94)#§ ^
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	24.7 (4.1)*	31.8 (7.2)§*
% predicted normative $\dot{V}O_{2peak}$¥	88.4 (10.1)	-
$\dot{V}O_{2peak}$ ($ml \cdot kg\text{-}ffm^{-1} \cdot min^{-1}$)	36.6 (4.3)*	44.2 (11.1)§*
Glucose baseline ($mmol \cdot l^{-1}$)†	6.07 (0.59)*	5.57 (0.55)*
Glucose OGTT 2 hour ($mmol \cdot l^{-1}$)	5.48 (1.89)	-
TG ($mmol \cdot l^{-1}$)†	1.57 (0.86)#	1.87 (1.15)#
HDL-c ($mmol \cdot l^{-1}$)†	1.34 (0.15)	1.28 (0.27)
LDL-c ($mmol \cdot l^{-1}$)†	3.69 (0.83)	3.42 (0.86)
Total cholesterol ($mmol \cdot l^{-1}$)†	5.87 (1.16)	5.58 (0.94)

Data are presented as mean and (SD). #Median (IQR). §Predicted value, see Swainson et al. (2019). ¥Predicted normative value calculated according to Wasserman et al. (2004) p.166. †Blood samples acquired in the fasted state. ‡Number of participants presenting with ≥ 3 risk factors indicative of the metabolic syndrome (MetS) (Alberti et al., 2009). *Independent t-test for mean or transformed data, $p < 0.05$. ^Mann-Whitney-U test $p < 0.05$. ∨Chi-square test of homogeneity, $p < 0.05$.

Table 6.2 continued Comparison of participant characteristics of individuals completing the screening visit and individuals with an FLI ≥ 60 from the Nuffield Health Screening Study (Thesis Chapter 4).

Variable	Screening visit	FLI ≥ 60 (high-risk FLI cohort within Chapter 4)
AST ($U \cdot l^{-1}$)†	27 (7)*	38 (10)*
ALT ($U \cdot l^{-1}$)†	34 (31)#	35 (20)#
GGT ($U \cdot l^{-1}$)†	28 (32)#	31 (24)#
FLI 30-59 (*n*, %)	2 (18.2%)	-
FLI ≥ 60 (*n*, %)	8 (72.7%)	127 (100%)
MetS (*n*, %)‡	8 (72.7%)	79 (62.2%)

Data are presented as mean and (SD). #Median (IQR). §Predicted value, see Swainson et al. (2019). ¥Predicted normative value calculated according to Wasserman et al. (2004) p.166. †Blood samples acquired in the fasted state. ‡Number of participants presenting with ≥ 3 risk factors indicative of the metabolic syndrome (MetS) (Alberti et al., 2009). *Independent t-test for mean or transformed data, $p < 0.05$. ^Mann-Whitney-U test $p < 0.05$. ∨Chi-square test of homogeneity, $p < 0.05$.

6.3.2 Supervised exercise training sessions

All participants completed the two supervised submaximal HIIE sessions. The mean (SD) power output (W) for the high-intensity and recovery intervals was 134 ± 12 W and 57 ± 10 W, respectively. Energy expenditure during exercise sessions, as estimated by the cycle ergometers performance monitor, was 286.2 ± 29.2 Kcal. The peak HR ($b \cdot min^{-1}$) attained during the exercise sessions was 91.1 ± 8.4% of that attained during CPET; and were not significantly different between the two visits (OFTT-Sucr-Ex: 141 ± 18 $b \cdot min^{-1}$; OFTT-Fruc-Ex: 136 ± 17; MD = 5 $b \cdot min^{-1}$, 95% CI = -3.0 to 12 $b \cdot min^{-1}$, $p = 0.187$, Cohen's $d = 0.71$). The peak RPE recorded during exercise sessions was 16 ± 2; and did not differ between exercise sessions (OFTT-Sucr-Ex: 16 ± 2; OFTT-Fruc-Ex: 16 ± 2; MD = 0.0, 95% CI = -1.0 to 1.0, $p = 0.621$, Cohen's $d = 0.24$).

6.3.3 Primary outcome

6.3.3.1 TG tAUC responses to OFTT

The TG tAUC responses are shown in Table 6.3 and Figure 6.2, respectively. The TG tAUC response was 0.78 mmol·4hr^{-1}·l^{-1} higher (95% CI = 0.31 to 1.26, p = 0.010, ηp^2 = 0.838) for the two fructose conditions compared to the two conditions in which sucrose was consumed. However, post hoc pairwise comparisons with Bonferroni correction failed to identify significant differences in the TG tAUC response when comparing OFTT-Fruc to OFTT-Sucr (MD = 0.78 mmol·4hr^{-1}·l^{-1}, 95% CI = -0.10 to 1.66, p = 0.068, ηp^2 = 0.605), or OFTT-Fruc-Ex to OFTT-Sucr-Ex (MD = 0.78 mmol·4hr^{-1}·l^{-1}, 95% CI = -0.09 to 1.65, p = 0.067, ηp^2 = 0.608), respectively. There was also a large effect size for a lower TG tAUC response following prior evening exercise compared to rest (MD = -1.13 mmol·4hr^{-1}·l^{-1}, 95% CI = -2.37 to 0.12, p = 0.067, ηp^2 = 0.610). There was no interaction effect between the experimental conditions on TG tAUC (p = 0.997, ηp^2 = 0.001).

6.3.4 Secondary outcomes

The postprandial responses for each of the secondary outcome measures below are shown in Table 6.3.

6.3.4.1 TG iAUC responses to OFTT and fasting concentrations

There was a large effect size for a higher TG iAUC response to the two fructose conditions compared to the two sucrose conditions (MD = 0.10 mmol·4hr^{-1}·l^{-1}, 95% CI = -0.14 to 0.33, p = 0.330, ηp^2 = 0.235). There was no difference in the TG iAUC response between rest and prior evening exercise (MD = 0.33 mmol·4hr^{-1}·l^{-1}, 95% CI = -0.88 to 1.55, p = 0.489, ηp^2 = 0.126). There was no interaction effect between interventions on the iAUC response (p = 0.986, ηp^2 = 0.001).

Fasting TG concentrations were 0.17 $mmol \cdot l^{-1}$ higher in the two fructose conditions compared to the two sucrose conditions (95% CI = 0.03 to 0.32, p = 0.031, $\eta p^2 = 0.729$). However, post hoc analysis with Bonferroni adjustment failed to identify significant differences in the fasting TG concentrations when comparing OFTT-Fruc to OFTT-Sucr (MD = 0.17 $mmol \cdot l^{-1}$, 95% CI = -0.26 to 0.60, p = 0.323, $\eta p^2 = 0.241$), or OFTT-Fruc-Ex to OFTT-Sucr-Ex (MD = 0.17 $mmol \cdot l^{-1}$, 95% CI = -0.10 to 0.44, p = 0.149, $\eta p^2 = 0.443$), respectively. There was also a large effect size ($\eta p^2 = 0.629$) for lowering of fasting TG concentrations following prior evening exercise compared to rest (MD = -0.20 $mmol \cdot l^{-1}$, 95% CI = -0.41 to 0.01, p = 0.060). There was no interaction effect between conditions on fasting TG concentrations (p = 0.987, $\eta p^2 = 0.001$).

6.3.4.2 Glucose and insulin tAUC responses to OFTT and fasting concentrations

There was a large effect size ($\eta p^2 = 0.358$) for a lower glucose tAUC response to the sucrose conditions compared to the fructose conditions (MD = -0.80 $mmol \cdot 4hr^{-1} \cdot l^{-1}$, 95% CI = -2.28 to 0.69, p = 0.210, Figure 6.2). There was no difference in the glucose tAUC response between rest and prior evening exercise (MD = 0.48 $mmol \cdot 4hr^{-1} \cdot l^{-1}$, 95% CI = -1.46 to 2.41, p = 0.532, $\eta p^2 = 0.105$). There was no interaction effect between interventions on glucose tAUC (p = 0.540, $\eta p^2 = 0.101$).

The insulin tAUC response was 26.52 $uIU \cdot 4hr^{-1} \cdot ml^{-1}$ lower in the two fructose conditions compared to the two sucrose conditions (95% CI = -43.74 to -9.30, p = 0.013, $\eta p^2 = 0.821$,Figure 6.2). However, post hoc pairwise comparisons with Bonferroni correction failed to identify significant differences in the insulin tAUC response when comparing OFTT-Fruc to OFTT-Sucr (MD = -21.52 $uIU \cdot 4hr^{-1} \cdot ml^{-1}$, 95% CI = -47.68 to 4.63, p = 0.084, $\eta p^2 = 0.566$), or OFTT-Fruc-Ex to OFTT-Sucr-Ex (MD = -31.51 $uIU \cdot 4hr^{-1} \cdot ml^{-1}$, 95% CI = -66.29 to 3.26, p = 0.066, $\eta p^2 = 0.613$), respectively. There was no difference

in the insulin tAUC response between rest and exercise conditions (MD = 9.94 uIU·4hr^{-1}·ml^{-1}, 95% CI = -48.32 to 68.20, p = 0.660, ηp^2 = 0.053). There was no interaction between the experimental conditions on the insulin tAUC response (p = 0.615, ηp^2 = 0.069).

There was a large effect size (ηp^2 = 0.489) for lower fasting glucose concentrations in the two exercise conditions compared to the two resting conditions (MD = -0.23 mmol·l^{-1}, 95% CI = -0.55 to 0.10, p = 0.122). There was no difference in fasting glucose concentrations between the sucrose and fructose conditions (MD = 0.13 mmol·l^{-1}, 95% CI = -0.27 to 0.53, p = 0.420, ηp^2 = 0.167). There was no interaction effect between interventions on fasting glucose concentrations (p = 0.618, ηp^2 = 0.068).

There were no differences in fasting insulin concentrations with prior evening exercise compared to rest (MD = 0.68 uIU·ml^{-1}, 95% CI = -3.32 to 4.70, p = 0.661, ηp^2 = 0.053), or between the sucrose and fructose conditions (MD = 0.63 uIU·ml^{-1}, 95% CI = -1.82 to 3.10, p = 0.513, ηp^2 = 0.114). There was no interaction between the experimental conditions on fasting insulin concentrations (p = 0.635, ηp^2 = 0.062).

6.3.4.3 TC and HDL tAUC responses to OFTT

There was a large effect size (ηp^2 = 0.577) for a lowering of the TC tAUC response in the two rest conditions compared to the two exercise conditions (MD = -1.323 mmol·4hr-^{1}·l^{-1}, 95% CI = -2.90 to 0.25, p = 0.080). There was no difference in the TC tAUC response between sucrose and fructose conditions (MD = 0.03 mmol·4hr-^{1}·l^{-1}, 95% CI = -1.36 to 1.96, p = 0.643, ηp^2 = 0.059). There was no interaction between the experimental conditions on the TC tAUC response (p = 0.532, ηp^2 = 0.105).

There was a large effect size (ηp^2 = 0.546) for a higher HDL tAUC response to the two sucrose conditions compared to the two fructose conditions (MD = 0.34 mmol·4hr-^{1}·l^{-1},

95% CI = -0.09 to 0.76, p = 0.093). There was no difference in the HDL tAUC response between the rest and exercise conditions (MD = 0.02 mmol·4hr-^{1}·l^{-1}, 95% CI = -0.36 to 0.39, p = 0.916, ηp^2 = 0.003). There was a large effect size for an exercise and fructose interaction effect (p = 0.168, ηp^2 = 0.413).

6.3.4.4 AST, ALT and GGT tAUC responses to OFTT

There was a large effect size (ηp^2 = 0.216) for a higher AST tAUC response to the two sucrose conditions compared to the two fructose conditions (MD = 43 U·4hr^{-1}·l^{-1}, 95% CI = -71 to 158, p = 0.353). A large effect size (ηp^2 = 0.516) for a higher AST tAUC response to the exercise compared to rest was also observed (MD = 19 U·4hr^{-1}·l^{-1}, 95% CI = -6 to 44, p = 0.108). There was no interaction effect between the interventions on AST tAUC (p = 0.505, ηp^2 = 0.118).

There was a large effect size (ηp^2 = 0.201) for a higher ALT tAUC response to the two sucrose conditions compared to the two fructose conditions (MD = 23 U·4hr^{-1}·l^{-1}, 95% CI = -41 to 87, p = 0.372). A large effect size (ηp^2 = 0.349) for a higher ALT tAUC response to the exercise compared to rest was also observed (MD = 23 U·4hr^{-1}·l^{-1}, 95% CI = -20 to 66, p = 0.217). There was no interaction effect between the experimental conditions on AST tAUC (p = 0.846, ηp^2 = 0.011).

There was a large effect size (ηp^2 = 0.168) for a higher GGT tAUC response to the two sucrose conditions compared to the two fructose conditions (MD = 12 U·4hr^{-1}·l^{-1}, 95% CI = -25 to 50, p = 0.420). There was no difference in GGT tAUC between rest and exercise conditions (MD = 2 U·4hr^{-1}·l^{-1}, 95% CI = -63 to 67, p = 0.939, ηp^2 = 0.002). There was a large effect size for an exercise and fructose interaction effect (p = 0.107, ηp^2 = 0.517).

Table 6.3 Baseline and postprandial metabolic responses to the mixed nutrient metabolic challenge for each study condition.

Variable	OFTT-Sucr	OFTT-Sucr-Ex	OFTT-Fruc	OFTT-Fruc-Ex	Effect size CHO type (ηp2)	p value CHO type	Effect size exercise (ηp2)	p value exercise
TG tAUC (mmol·4hr^{-1}·l^{-1})	9.16 (1.91)	8.04 (1.40)	9.94 (1.97)*	8.82 (1.86)*	0.838	0.010*	0.610	0.067
TG iAUC (mmol·4hr^{-1}·l^{-1})	3.64 (0.98)	3.30 (0.42)	3.73 (0.98)	3.40 (1.19)	0.235	0.330	0.126	0.489
TG baseline (mmol·l^{-1})	1.38 (0.34)	1.18 (0.38)	1.55 (0.50)	1.35 (0.36)	0.729	0.031	0.629	0.060
Glucose tAUC (mmol·4hr^{-1}·l^{-1})	22.84 (3.17)	22.61 (2.79)	23.88 (2.73)	23.16 (1.90)	0.358	0.210	0.105	0.532
Glucose baseline (mmol·l^{-1})	6.21 (0.96)	5.90 (0.76)	6.25 (0.57)	6.11 (0.75)	0.167	0.420	0.489	0.122
Insulin tAUC (uIU·4hr^{-1}·ml^{-1})#	92.00 (153.0)	120.25 (118.68)	58.48 (184.9)*	63.22 (131.04)*	0.821	0.013*	0.053	0.660
Insulin baseline (uIU·ml^{-1})#	11.78 (14.52)	8.10 (13.99)	9.95 (9.23)	11.22 (14.04)	0.114	0.513	0.053	0.661
TC tAUC (mmol·4hr^{-1}·l^{-1})#	20.19 (4.40)	20.31 (2.74)	19.64 (1.95)	20.84 (2.33)	0.059	0.643	0.577	0.080
HDL tAUC (mmol·4hr^{-1}·l^{-1})	5.58 (0.85)	5.42 (0.68)	5.07 (0.80)	5.26 (0.43)	0.546	0.093	0.003	0.916

Data are presented as mean (SD). #Denotes median (IQR). CHO; carbohydrate. Effect size calculated as Partial Eta Squared (ηp^2). *Statistically significant main effect for carbohydrate type.

Table 6.3 continued Baseline and postprandial metabolic responses to the mixed nutrient metabolic challenge for each study condition.

Variable	OFTT-Sucr	OFTT-Sucr-Ex	OFTT-Fruc	OFTT-Fruc-Ex	Effect size CHO type (ηp^2)	p value CHO type	Effect size exercise (ηp^2)	p value exercise
AST tAUC ($U \cdot 4hr^{-1} \cdot l^{-1}$)#	126 (175)	130 (158)	94 (71)	119 (80)	0.216	0.353	0.516	0.108
ALT tAUC $U \cdot 4hr^{-1} \cdot l^{-1}$)#	130 (141)	155 (191)	131 (127)	157 (140)	0.201	0.372	0.349	0.217
GGT tAUC $U \cdot 4hr^{-1} \cdot l^{-1}$)#	86 (231)	85 (126)	58 (164)	92 (153)	0.168	0.420	0.002	0.939

Data are presented as mean (SD). #Denotes median (IQR). CHO; carbohydrate. Effect size calculated as Partial Eta Squared (ηp^2). *Statistically significant main effect for carbohydrate type.

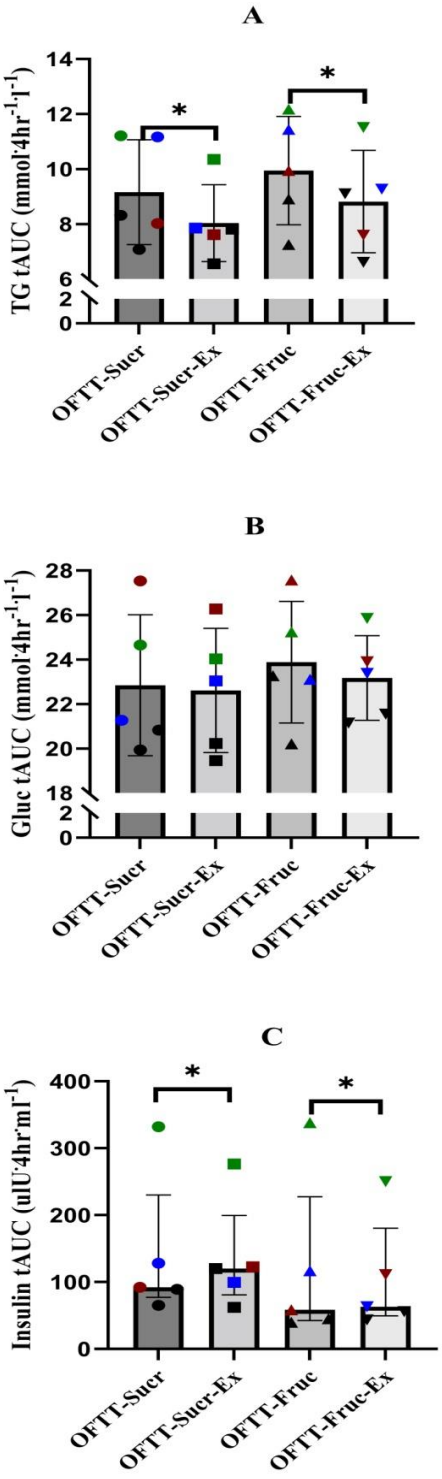

Figure 6.2 Postprandial triglyceride (TG), glucose (gluc) and insulin responses to each study condition. Panel A: Mean (SD) TG tAUC; panel B: Mean (SD) gluc tAUC; and panel C: Median (IQR) insulin tAUC responses to the respective study conditions. Each data point represents the individual response to each condition, respectively. Red, blue and green data points represent those participants with MetS and FLI ≥ 60. *Denotes a statistically significant main effect for carbohydrate type ($p < 0.05$).

6.4 Discussion

The current study investigated the postprandial metabolic responses to an oral fat load with the inclusion of an energy matched sucrose or fructose fraction, preceded by rest or prior evening exercise, among inactive, overweight and centrally obese males. Combined fat and fructose ingestion exacerbated the postprandial TG tAUC response compared to an iso-caloric fat and sucrose load. A large effect size was observed for lowering of the TG tAUC response with prior evening exercise. Secondary outcome measures included a lower insulin response to fructose compared to sucrose combined within the OFTT. A large effect size was also apparent for a lower glucose tAUC response to sucrose in comparison to fructose inclusion with the OFTT. Prior exercise did not further attenuate postprandial insulin or glucose responses.

Khalafi et al. (2022) in a recent meta-analysis provides contemporary evaluation of the effects of HIIE as compared to moderate intensity exercise (MIE) and control conditions on postprandial glucose and insulin responses. In total, 30 studies comprising 36 intervention arms and involving 350 adult participants were considered. HIIE reduced postprandial glucose and insulin responses compared with control conditions. Based on subgroup analyses, these reductions were significant for healthy adult participants, with larger effects noted amongst participants with metabolic disorders. However, when comparing studies matched for total work performed, HIIE was more effective for decreasing postprandial as compared with MIE. In addition, HIIE has superior effects for reducing postprandial TGs as compared with MIE, when equivalent work was performed at both intensity levels.

6.4.1 Fructose compared to sucrose consumption and postprandial metabolic responses

It is well established that fructose and fructose containing sugars augment the postprandial TG response to an oral fat challenge (Cohen & Schall, 1988; Grant et al., 1994; Singleton et al., 1999). Although others have compared fructose and sucrose co-ingestion with fat in apparently healthy individuals (Cohen & Schall, 1988; Gallagher et al., 2016; Saito et al., 2015), the current study is the first, to the best of our knowledge, to delineate the acute postprandial responses to fructose versus sucrose ingestion with dairy fat in inactive, overweight and centrally obese adult males.

The increased TG tAUC and lower insulin tAUC responses to oral fructose and fat feeding are consistent with previous observations (Chong et al., 2007). The reduced insulinaemic response has been cited as a mechanism for the acute effects of fructose in augmenting postprandial TG excursions (Chong et al., 2007). Specifically, the insulin independent metabolism of fructose limits the activation of LPL and reduces the clearance of TGs into adipose tissue (Chong et al., 2007). The suppression of NEFA release from adipose tissue and blunting of hepatic VLDL-TG synthesis are also reduced with lower insulin concentrations (Frayn et al., 1993; Jensen et al., 1989; Lewis et al., 1995). Acute fructose ingestion is also know to stimulate hepatic DNL (Chong et al., 2007; Hengist et al., 2019) as well as intestinal TG production within the enterocyte (Hoffman et al., 2019; Steenson et al., 2020); both of which may be increased several-fold in individuals with poor metabolic health (Lambert et al., 2014; Steenson et al., 2020).

In contrast to our findings, Gallagher and colleagues (2016) reported no differences in the postprandial TG response to fructose compared to sucrose inclusion within an OFTT in a cohort that included overweight and obese adult males. This was despite similarly observing a significantly lower insulin response to fructose ingestion. There are however

several methodological disparities between the studies that may explain these conflicting findings. For example, in the current investigation, both metabolic challenges were matched for energy density and provided 60 g of either fructose or sucrose, respectively, in addition to a recommend oral fat load (75 g) (Kolovou et al., 2019a). Conversely, Gallagher and associates (2016) provided 52 g of fructose or 65 g of sucrose combined with a 66 g fat load. The author's rationale for not matching meals for energy content was to maintain palatability and blinding. The methods of delivering the meals also differed in terms of their physical structure (liquid versus solid). Perhaps the most notable difference was the study populations. Our relatively homogenous group of five overweight, centrally obese, inactive males, three of whom satisfied the criteria for MetS (Alberti et al., 2009), was different to the heterogeneous group of apparently healthy males and females recruited by Gallagher and co-workers (2016). Indeed, in apparently healthy volunteers, others have documented no differences in TG or insulin excursions when combined fructose and fat or sucrose and fat solutions are matched for energy content (Saito et al., 2015). Our data therefore support the suggestion that fructose consumption exacerbates postprandial lipaemia in the context of positive energy balance and/or low energy turnover (Wang et al., 2014; Hengist et al., 2019; Tappy & Rosset, 2019). Matikainen et al. (2017) were the first to report the effect of fructose consumption in obese non-diabetic males on metabolic responses following both an OGTT and a mixed meal. These investigators showed that despite mild but significant weight and liver fat gain accompanied with fasting and postprandial TG increase, fructose consumption had no impact on glycaemic control.

From a practical perspective, our findings demonstrate that when fructose is consumed as sucrose in a mixed nutrient meal, as it is most frequently in free-living conditions, that sucrose produces less pronounced, more favourable metabolic excursions to that of fructose alone. This may be explained by the insulinaemic response to sucrose because

of its glucose fraction (Chong et al., 2007). Our data show a significantly higher insulin response to sucrose and fat compared to a fructose and fat load, coupled with a lower TG response. In accordance with the aforementioned mechanisms, insulin action potentiates greater LPL activation and TG uptake into adipose tissue (Chong et al., 2007; Sadur & Eckel, 1982), in addition to suppressing both NEFA release and synthesis of hepatic VLDL-TG (Frayn et al., 1993; Jensen et al., 1989; Lewis et al., 1995). A large effect was also apparent for a lower glucose tAUC response to sucrose in comparison to fructose inclusion within the OFTT. Collectively, these results contradict previous findings in which lower insulin, glucose, and/or TG concentrations have been documented following the inclusion of fructose, as opposed to glucose or glucose containing carbohydrates, within a mixed meal (Evans et al., 2017b; Gallagher et al., 2016). This had led some to conclude that the iso-caloric replacement of glucose or sucrose with fructose may be beneficial to those living with T2DM (Evans et al., 2017b; Gallagher et al., 2016), although these benefits may be limited (Evans et al., 2017a). These equivocal findings are likely related to the methodological differences between studies, including the dose and structure in which fructose was provided, the duration of dietary interventions, and sex differences (Chong et al., 2007; Jang et al., 2020; Pinnick & Hodson, 2019; Tran et al., 2010).

Our data therefore support the suggestion that acute fructose consumption combined with an oral fat load exacerbates postprandial TG responses in inactive, overweight, and centrally obese adult males. The ingestion of sucrose, the primary method of fructose intake in free-living settings, confers less pronounced metabolic excursions within this context.

6.4.2 Exercise and postprandial metabolic responses to fructose and sucrose

There was a large effect size for a reduction in TG tAUC by approximately 12% following submaximal HIIE compared to the rest conditions. Large effect sizes were also observed for lower fasting TG and glucose concentrations following prior evening exercise. Although not statistically significant in the current investigation, reductions in TG tAUC of a similar magnitude the morning after exercise have been reported by others (Burns et al., 2015; Maraki & Sidossis, 2013). Alternatively, no significant differences in fasting TG between 30 or 60 minutes of MIE (60 % $\dot{V}O_{2peak}$) compared to the control condition were evident in 12 overweight, insufficiently active men, performed 12 hours prior to a high-fat meal (Emerson et al., 2016). Similarly there were no differences in the tAUC, iAUC, peak or time-to-peak between trials for the TG response (Emerson et al., 2016). O'Doherty et al. (2017) investigated whether acute submaximal HIIE improved postprandial responses to an OFTT in ten overweight/obese males (age: 31.5 years; BMI: 29.9 ± 1.8 $kg \cdot m^{-2}$). The TG tAUC was significantly (1.5 $mmol \cdot 4hr^{-1} \cdot l^{-1}$) lower for the exercise conditions compared with the resting (control) conditions (95% CI: −2.3 to −0.8 $mmol \cdot 4hr^{-1} \cdot l^{-1}$). However, few studies have examined the potential benefits of acute exercise on the postprandial responses to concomitant fat and fructose ingestion (Macedo et al., 2019; Rowe et al., 2016; Wilburn et al., 2015).

Macedo and colleagues (2019) successfully reduced postprandial TG excursions to an oral fat and fructose load following 45 minutes of continuous exercise at 60% $\dot{V}O_{2peak}$. However, the reported ~ 30% reduction in the TG tAUC response was of a significantly greater magnitude to that of our study. In the current investigation, submaximal HIIE was prescribed according to exercise intensity domains identified by expired ventilatory gas analysis measured during a CPET (O'Doherty et al., 2017; Özyener et al., 2001). This method of exercise prescription, referred to as the threshold-based approach, has several advantages compared to tradition models of programming exercise interventions at

percentages of $\dot{V}O_{2max}$ (Mezzani et al., 2013; Wolpern et al., 2015). Briefly, exercise prescribed according the AT and critical power ensured that the relative exercise intensity was similar for each participant and so minimising the inter- and intra-individual response to the exercise conditions (Wolpern et al., 2015). Although other methodological differences must be considered, the disparity in the magnitude of the postprandial TG reductions between our study and that of Macedo and associates (2019) may therefore be attributed, at least in part, to the contrasting methods of exercise prescription.

It is important to note that previous studies have recruited apparently healthy participants (Macedo et al., 2019; Rowe et al., 2016; Wilburn et al., 2015) compared to the cohort of inactive, overweight and centrally obese males in the present investigation. From a practical perspective, HIIE has several advantages that may be pertinent to our recruited participants, including higher levels of enjoyment, lower perceived exertion and increased likelihood of exercise adherence (Heinrich et al., 2014; Martinez et al., 2015). Interval exercise also allows for a greater amount of energy to be expended in a time efficient manner (Trombold et al., 2013). This is a key consideration as a lack of time is a cited as common barrier to exercise participation (Korkiakangas et al., 2009; Reichert et al., 2007), whilst previous studies have suggested that exercise energy expenditure may determine the extent of the reduction in postprandial lipaemia (Freese et al., 2014). Although exercise energy expenditure was estimated to be 286.2 ± 29.2 Kcal in the current study (based on calculations from the cycle ergometer), this was not quantified objectively as equations for calculating energy expenditure by indirect calorimetry may be invalidated at exercise intensities above the AT (Jeukendrup & Wallis, 2005). A further consideration of this method is that exercise should attain a steady state (Jeukendrup & Wallis, 2005). The high-intensity and intermittent nature of the exercise protocol used in our study would have therefore invalidated these assumptions (O'Doherty et al., 2017).

In contrast, Rowe and colleagues (2016) asked participants to walk continuously at 70% $\dot{V}O_{2max}$ until they had expended 500 Kcal. Despite the significantly greater energy displacement, almost double that estimated in our study, no differences were reported in the postprandial TG response to a mixed fructose meal with and without prior evening exercise. As highlighted by Burns and associates (2015), estimated energy expenditure appears to be less during HIIE interventions that have successfully lower postprandial TG excursions compared to when moderate intensity continuous exercise is prescribed. Moreover, when matched for estimated energy expenditure, HIIE has been shown to reduce postprandial TGs to a greater extent than MIE in healthy recreationally active young men (n = 6) with moderately high cycle ergometry $\dot{V}O_{2peak}$: 55.5 ± 1.3 $ml \cdot kg^{-1} \cdot min^{-1}$ (Trombold et al., 2013). In the above study, compared with a control condition, both MIE (50% $\dot{V}O_{2peak}$ for 60 minutes), or isoenergetic HIIE (alternating 2 minutes at 25% and 2 minutes at 90% $\dot{V}O_{2peak}$) significantly attenuated postprandial TG concentrations (iAUC; 75.2% ± 15.5%, and 54.9% ± 13.5% respectively; with HIIE also significantly lower than MIE). Total energy expenditure during the exercise trials, after correction for resting measurements, was reported not different between the acute exercise interventions (660.5 ± 35.7 Kcal and 654.8 ± 30.6 Kcal). The average rate of oxygen consumption was significantly lower in MIE (2.19 ± 0.28 $l \cdot min^{-1}$, 48.8% ± 1.2% $\dot{V}O_{2peak}$) compared with HIIE (3.34 ± 0.5 $l \cdot min^{-1}$, 74.7% ± 6.1% $\dot{V}O_{2peak}$). These observations suggest that a mechanism in addition to energy expenditure may have been responsible for the large effect size for reducing TG tAUC in our study. It has been suggested that LPL activity is muscle fibre specific and may increase with HIIE because of the greater recruitment of type 2 muscle fibres (Burns et al., 2015; Gabriel et al., 2013). This mechanism would therefore allow for greater TG clearance. It is also possible that this mechanism is responsible for the reductions in postprandial TGs with MIE as type 2 muscle fibre recruitment increases with exercise duration (O'Doherty et al., 2017). Indeed,

recent data has shown that the insulin sensitising effect of exercise is similar in both type 1 and type 2 human muscle fibres following a single bout of exercise (Larsen et al., 2020). Both exercise intensity and duration are associated with energy expenditure, therefore greater energy expenditure may simply be an artefact, as opposed to a cause, of the reduction in postprandial TGs with acute exercise.

Our data therefore support the prescription of submaximal HIIE to attenuate postprandial TG excursions in response to oral fat and fructose feeding in inactive, overweight, and centrally obese men.

6.4.3 Strengths and limitations

As alluded to above, the present investigation has several strengths to its design, including the use of energy matched mixed nutrient metabolic challenges in which the fat content conformed to guidelines aimed to standardise the OFTT. The sucrose and fructose fractions were also reflective of habitual consumption and previous literature, respectively. The intensity of the exercise interventions was also prescribed in a robust, individualised manner to reduce the inter- and intra-individual responses.

It is also important to appraise the limitations of the current study. As noted above, the failure to reach the recruitment target of ten participants means that our findings should be interpreted with caution given the lack of statistical power to detect a significant change in the primary outcome variable of TG tAUC on which the sample size estimation was calculated. Unfortunately, data collection was terminated due to restrictions imposed to limit the Covid-19 pandemic. At the time, five participants had completed the study with a further three that had met the inclusion criteria following successful screening visits (see Figure 6.1).

Missing data points and blood sampling frequency are also further considerations. Although 90% of blood samples were collected successfully, missing data is not uncommon in time series data as the probability of error is increased when a large number of samples are collected (Narang et al., 2020). However, the methods for dealing with missing data can be varied and ambiguous. We utilised mean imputation (pooled mean of data points either side of the missing value) to account for missing samples (Watkins et al., 2020). Ideally, participants would have been cannulated to reduce the number of venepunctures and possibility of missed samples. Cannulation would have also allowed for a greater number/frequency of blood samples to better capture the postprandial response to the study interventions. If the study was to be repeated, the addition of an OFTT control without the inclusion of fructose or sucrose would allow for the independent and combined effects of carbohydrate type and exercise to be investigated.

6.4.4 Summary and conclusions

In summary, oral fat and fructose ingestion exacerbated the postprandial TG response compared to an energy matched fat and sucrose load. Acute submaximal HIIE may be an effective strategy to attenuate the TG tAUC response in inactive, overweight, centrally obese males and therefore reduce the risk of cardiometabolic disorders, including hepatic steatosis. Future studies are needed to further elucidate the lipogenic potential of fructose, including mechanisms with the liver and gut. On the basis of these findings, further research is required on the efficacy and effectiveness of higher-intensity acute exercise within patients with clinically defined NAFLD and biomarkers of advanced hepatic fibrosis.

Chapter 7 General discussion

7.1 Overview and main findings

The aims of the current thesis were:

1. To determine the prevalence of NAFLD and hepatic fibrosis in an unselected cohort of apparently healthy young to middle-aged adults using several minimally invasive composite panels. Further analysis was undertaken to examine the association between predictors of metabolic risk, hepatic steatosis and fibrosis.
2. To investigate if acute HIIE could attenuate the postprandial responses to a mixed nutrient metabolic challenge comprised of a recommended oral fat load with the inclusion of fructose or sucrose in apparently healthy men.
3. To investigate if acute HIIE could attenuate the postprandial responses to a mixed nutrient metabolic challenge comprised of a recommended oral fat load with the inclusion of fructose or sucrose in inactive, overweight, and centrally obese men.

These aims were addressed using observational data and two acute experimental studies. The first study (Chapter 4) utilised several proxy scoring panels to determine the prevalence of hepatic steatosis and fibrosis in a cohort of apparently healthy young to middle-aged adults. This is important as composite panels offer a simple and inexpensive method to screen patients in primary care settings where NAFLD is under-recognised and often an incidental finding (Alexander et al., 2018; Armstrong et al., 2012). From a research perspective, the use of these panels could permit the population-based study of NAFLD and hepatic fibrosis (Marchesini et al., 2016). The study was novel in that we sought to determine the prevalence of hepatic steatosis and risk of fibrosis based exclusively on minimally invasive scores. Using the FLI (Bedogni et al., 2006), the predicted prevalence of NAFLD was 34.9% in our cohort and comparable to previous

estimates for the UK adult population (~ 33% as determined by ultrasound methods), as well as the global estimated prevalence (30%) (Abeysekera et al., 2020; Armstrong et al., 2012; Younossi et al., 2023). Although not determined in the current thesis, others have validated the FLI against MRS as the criterion measure and advocated its use for screening in clinical practice and research settings (Cuthbertson et al., 2014). Conversely, the prevalence/risk of hepatic fibrosis varied significantly according to the complexity of the composite panel and cut-off applied. These findings were consistent with the observations of others (Long et al., 2016) and suggest that complex as opposed to simple panels provide a more accurate estimate of hepatic fibrosis (Adams et al., 2011; Xiao et al., 2017). However, composite panels for the determination of fibrosis should be interpreted by a specialist in liver disease according to the clinical context and accounting for the findings of other investigations (EASL-ALEH, 2015).

Further analysis was performed to explore the associations between established risk factors for NAFLD, liver fat and fibrosis. In our cohort, structural equation models identified that adiposity as indicated by BMI and WC were associated with an increased risk of advanced fibrosis as determined by the NFS (Angulo et al., 2007). These observations remained consistent when sex, smoking status, alcohol intake and physical activity were introduced to the model. Furthermore, our analysis identified MetS as a significant predictor of liver fat. Collectively, these findings add support to previous observations that the FLI may be used as a minimally invasive and inexpensive screening tool to determine the presence of hepatic steatosis in primary care and research settings. In addition, the combination of BMI and WC are simple anthropometric measures that may be useful in identifying those with an increased risk of developing hepatic fibrosis. These findings were used to inform the inclusion and exclusion criteria for the distinct acute intervention studies and examine the postprandial responses to acute physical exercise interventions.

The aim of the first intervention study (Chapter 5) was to investigate the acute effects of exercise and fructose on postprandial metabolic responses in apparently healthy, recreationally active individuals. Poor lipid and glucose handling following meal ingestion is an independent risk factor for obesity and associated disease states (Berry et al., 2020; Blaak et al., 2012; Kolovou et al., 2011). Similarly, fructose has received increasing amounts of research attention and has been linked to the development of NAFLD (Tappy, 2018; Taskinen et al., 2017). Pronounced postprandial lipaemic responses to an OFTT is a well-established, and some would contend, independent risk factor for cardiometabolic diseases (Blaak et al., 2012; Kolovou et al., 2011); and is further accentuated with the addition of fructose or fructose containing sugars (Cohen & Schall, 1988; Grant et al., 1994). Prior evening exercise is a strategy to reduce both postprandial lipid and glucose excursions the following morning (Freese et al., 2013; O'Doherty et al., 2017). However, data pertaining to the efficacy of HIIE is limited (Burns et al., 2015). Similarly, the amount of fructose included in the majority of existing studies utilising mixed nutrient metabolic challenges is not representative of habitual intake (SACN, 2015). Instead, fructose is primarily consumed as sucrose in the real world setting (SACN, 2015). A direct comparison between the acute metabolic effects of fructose and sucrose combined within an OFTT was therefore of interest. In accordance with the findings of Chapter 4, participants with a BMI $\geq$ 30 kg·m^{-2} and/or a WC $\geq$ 94 cm were excluded from the study as apparently healthy active adult males were the participants of focus. Subsequently, none of the participants met the criteria for MetS or NAFLD (FLI $\geq$ 60).

The primary findings of Chapter 5 demonstrated that fructose combined with the OFTT significantly increased the TG iAUC compared to an energy matched sucrose and fat solution. This was despite comparable insulin and glucose responses between the two interventions. Previous data have proposed that the lower insulinaemic response typically

observed following fructose ingestion has the potential to increase TG concentrations (Chong et al., 2007). Our data therefore suggest that a further mechanism, in addition to or independent of insulin, may be responsible for the pronounced TG excursions in apparently healthy individuals. Although yet to be fully elucidated, fructose has the potential to increase TG concentrations by hepatic and intestinal DNL (Low et al., 2018; Steenson et al., 2020; Theytaz et al., 2014). Contemporary data has also shown considerable inter-individual differences in postprandial metabolic responses to the same meals even in highly adherent participants (Berry et al., 2020). Furthermore, participants may have found it challenging to replicate their physical activity and nutritional habits prior to each visit (Chrzanowski-Smith et al., 2020); this is despite clear verbal and written instructions regarding the study protocol and a standardised evening meal being provided prior to all experimental visits.

Although considered a strength of the study design, the provision of an evening meal and the subsequent replenishment of hepatic and muscle glycogen stores may have also attenuated the metabolic benefits of the acute exercise interventions (Johnson-Bonson et al., 2021; Taylor et al., 2018). Indeed, we observed no differences in the postprandial TG response to OFTT with the inclusion of fructose or sucrose with prior evening exercise. Burns et al. (2015) have systematically reviewed the literature on the effects of acute HIIE on postprandial TG concentrations. Fifteen studies were identified, in which the effect of interval exercise (intensity of > 65% of $\dot{V}O_{2peak}$ including either supramaximal exercise or submaximal interval exercise) was evaluated on postprandial TG levels. Ten studies examined the effect of a single session of low-volume HIIE including supramaximal sprints on postprandial TG. Seven of these studies noted reductions in the postprandial TG tAUC the morning after exercise of between 10% and 21% compared with resting conditions. However, three investigations found no significant difference in TG levels. Variations in the HIIE protocol used, inter-individual variation or insufficient time post-

exercise for an increase in LPL activity were highlighted as proposed reasons for the divergent results among studies. Five studies examined the effect of high-volume submaximal interval exercise on postprandial TGs. Four of these studies were characterised by high exercise energy expenditure and effectively attenuated total postprandial TG levels by ~ 15–30%, but one study with a lower energy expenditure found no effect on TGs. The evidence suggests that supramaximal HIIE can induce large reductions in postprandial TG levels, but findings are inconsistent. However, further studies are needed to delineate not only the effect of energy replacement *per se*, but also the macronutrient composition and the proximity of meals to both exercise and the nutritional metabolic challenge to determine the impact on postprandial metabolic responses.

Having observed the lipogenic effect of fructose in apparently healthy active participants, this work was extended in Chapter 6 to include those with risk factors for MetS and NAFLD. As informed by the outcomes of Chapter 4, inactive male volunteers with a BMI $\geq$ 25.0 $kg{\cdot}m^{-2}$ and a WC $\geq$ 94 cm were recruited to the study. These inclusion criteria resulted in a study cohort with an FLI of 72 (27) (mean and SD) indicative of suspected NAFLD (Bedogni et al., 2006), of which 3/5 also satisfied the criteria for MetS (Alberti et al., 2009).

The primary findings of Chapter 6 showed that combined fat and fructose ingestion exacerbated the postprandial TG tAUC response compared to an iso-caloric fat and sucrose load within inactive overweight males. A large effect size was observed for lowering of the TG tAUC response with prior evening exercise. It must be noted that these results should be interpreted with caution as the *a priori* sample size estimation was not achieved because of restrictions related to the COVID-19 pandemic. Nevertheless, these findings add further support to the suggestion that fructose consumption exacerbates postprandial lipaemia, particularly in the context of positive energy balance and/or low

energy turnover (Hengist et al., 2019; Tappy & Rosset, 2019), i.e. inactive, overweight or centrally obese individuals at risk of cardiometabolic disease, including NAFLD. Submaximal HIIE reduced the TG tAUC by approximately 12% compared to the rest conditions and could therefore be an effective strategy to attenuate TG excursions following high fat, high carbohydrate meals in those with poor metabolic health. Submaximal interval exercise appears to offer no TG metabolic or time advantage over continuous aerobic exercise but is a suitable alternative form of exercise to attenuate postprandial TG metabolism. The continuation of this work to include participants with clinically defined NAFLD warrants further investigation.

7.2 Strengths and limitations

The strengths and limitations of each study have been discussed in the respective chapters. However, there are considerations specific to the two acute experimental studies (Chapter 5 and Chapter 6) given the similarities in the methodological design. The strengths of these studies include the single-blind, randomised, repeated measures design, in which each participant served as their own control. This could have been made further robust by using a double-blind design and would be simple to implement from a practical perspective by asking a technician or other non-study staff member to prepare the OFTT solutions. The non-proprietary OFTT solutions have been used previously in our laboratory (O'Doherty et al., 2017, 2018) and were specifically designed to meet expert panel guidance on postprandial lipid testing for determining cardiometabolic risk (Kolovou et al., 2011). The isocaloric addition of habitual amounts of sucrose or fructose comparative to previous studies for the specific aims of the current thesis allowed for comparisons between the study conditions in the absence of disparities in energy content. With hindsight, a further study arm to include the OFTT solution alone would have been beneficial to delineate the metabolic effects of sucrose and fructose in addition to the OFTT. However, participants were required to attend the laboratory on seven occasions

in total including screening and evening exercise visits; therefore, further sessions may have become a potential barrier to study recruitment.

An additional strength was the use of a standardised evening meal prior to study visits the following morning. Previous studies have shown that this is an important consideration as the macronutrient content and replenishment of energy/glycogen stores subsequently affects both fasting and postprandial metabolic responses the following morning (Estafanos et al., 2022; Johnson-Bonson et al., 2021). Although participants were asked to replicate their dietary intake in the 24 hours prior to each study visit, no method of recording such as a food diary or dietary recall were utilised. Participants were also asked to refrain from exercise the day prior to study visits (not including supervised exercise sessions); however, no data was collected to quantify adherence. The use of an activity monitor and other objective methods should be a consideration if the current work was to be extended. Finally, an overreaching strength of these studies was that all study procedures (CPET interpretation and subsequent exercise prescription; making the OFTT; blood collection, processing, and storage, amongst others) were performed consistently by the same investigator (Mr Richard Page).

7.3 Future directions

The future directions of this work would include its extension into groups with clinically defined NAFLD to further investigate the acute effects of fructose feeding and exercise on postprandial responses. This would provide a continuous thread from apparently healthy individuals to those with risk factors of/with MetS known to precede cardiometabolic disease, and finally individuals living with confirmed NAFLD. The addition of sophisticated methods, such as advanced imaging (MRS), labelled isotopes, and muscle/adipose tissue biopsies, would help further delineate the underlying mechanisms linking nutritional and exercise interventions to the observed changes in

postprandial responses. A further line of enquiry could be to explore postprandial responses under free-living conditions (Berry et al., 2020). This would not only increase the ecological validity of the study but also remove potential barriers to recruitment such as repeated laboratory visits, and subsequently increase the sample size for a sufficiently powered study. This could also allow for the longitudinal effects of fructose ingestion, interval exercise, or both to be investigated as part of a chronic intervention study. Finally, the intervention studies in this thesis and the majority of empirical data are based on observations from adult male participants (Freese et al., 2013, 2014; Smith et al., 2022). Further consideration should be given to the design of future studies to include more female participants or indeed female only studies. Addressing this sex imbalance within exercise-related research and further our understanding of the prevention of cardiometabolic disorders and hepatic steatosis within females remain an important issue.

7.4 Conclusions

The findings of this thesis highlight the utility of proxy scoring panels and simple anthropometric measures in self-selected, apparently healthy individuals to identify those at risk of NAFLD and hepatic fibrosis. Specifically, BMI and WC may be used concurrently with routine biochemical measures, including LFTs, to rapidly screen and identify those at higher risk of metabolic/hepatic disease progression and can be easily incorporated into the primary care setting. NAFLD is an increasingly common but under-recognised condition and often presents as an incidental finding within preventive assessments of the apparently healthy, especially overweight/obese and relatively inactive young and middle-aged adults. The two acute intervention studies further highlight the lipogenic effects of fructose ingestion within both apparently healthy individuals and those at risk of cardiometabolic disease, including suspected NAFLD. Importantly, submaximal HIIE without significant energy deficits may reduce postprandial TG excursions and therefore attenuate cardiometabolic risk in inactive, overweight, centrally

obese males. The prescription of submaximal HIIE offers an additional strategy that may be incorporated into weekly or daily exercise routines to reduce the risk of postprandial dyslipidaemia and risk of cardiometabolic and hepatic disorders in both young and aging adults.

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Appendix 1 Ethical approval for Chapter 5

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PRIVATE AND CONFIDENTIAL
Richard Page
Faculty of Health Sciences
University of Hull
Via email

22nd October 2018

Dear Richard

REF FHS83 - Postprandial metabolic responses to high fat, high carbohydrate meals with and without prior exercise in apparently healthy men.

Thank you for your application to the Faculty of Health Sciences Research Ethics Committee.

Given the information you have provided I confirm approval by Chair's action.

Please refer to the Research Ethics Committee web page for reporting requirements in the event of any amendments to your study.

I wish you every success with your study.

Yours sincerely

Professor Liz Walker
Chair, FHS Research Ethics Committee

UNIVERSITY OF HULL

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Appendix 2 Ethical approval for Chapter 6

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PRIVATE AND CONFIDENTIAL
Richard Page
Faculty of Health Sciences
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Via email

12th November 2019

Dear Richard

REF FHS161 - The effects of carbohydrate type and acute exercise on postprandial responses to a metabolic challenge in centrally overweight/obese males. Form C: Notice of Substantial Amendment 8th November 2019.

Thank you for submitting your ethics Form C: Notice of Substantial Amendment to the Faculty of Health Sciences Research Ethics Committee.

Given the information you have provided I confirm approval by Chair's action.

Please refer to the Research Ethics Committee web page for reporting requirements in the event of any amendments to your study.

I wish you every success with your study.

Yours sincerely

Dr Tim Alexander
Deputy Chair, FHS Research Ethics Committee

UNIVERSITY OF HULL

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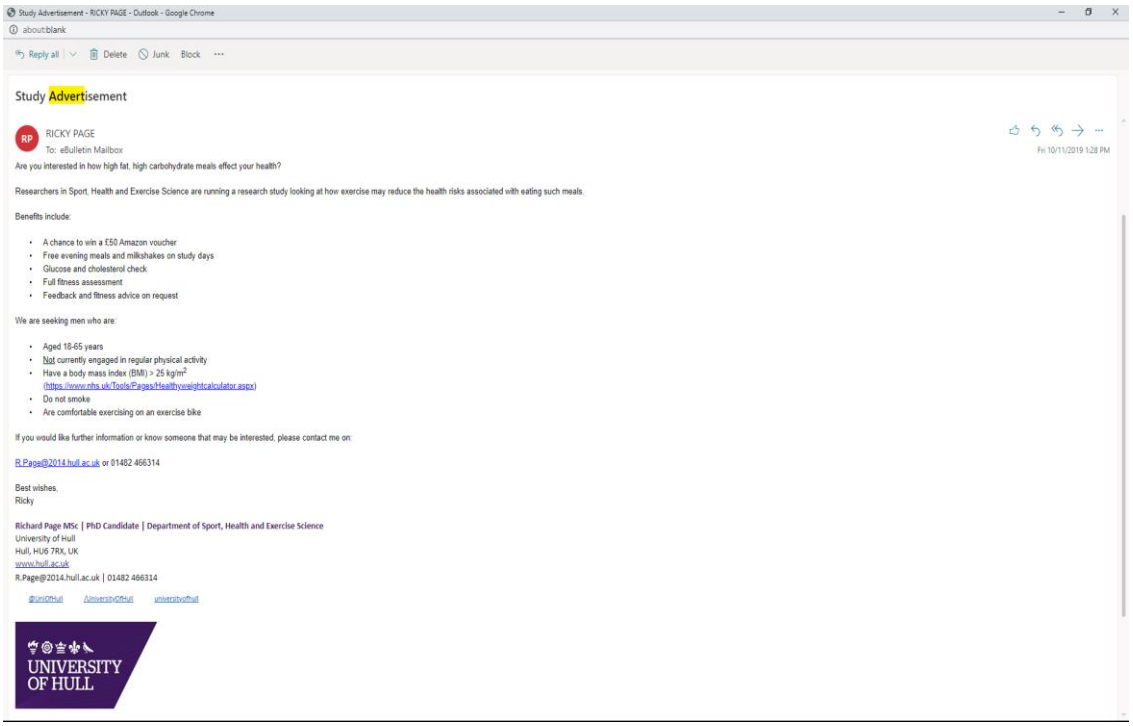
Appendix 3 Poster for Chapter 6

Appendix 4 Email advert for Chapter 6



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- Not currently engaged in regular physical activity
- Have a body mass index (BMI) > 25 kg/m^2 (https://www.nhs.uk/Tools/Pages/Healthyweightcalculator.aspx)
- Do not smoke
- Are comfortable exercising on an exercise bike

If you would like further information or know someone that may be interested, please contact me on:

R.Page@2014.hull.ac.uk or 01482 466314

Best wishes,
Ricky

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