

THE UNIVERSITY OF HULL

THE EFFECTS OF ACUTE EXERCISE AND NUTRITIONAL  
INTERVENTIONS ON POSTPRANDIAL LIPID METABOLISM

A Thesis submitted for the Degree of  
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## Abstract

Raised postprandial triglycerides (TG) is an independent risk factor for cardio-metabolic disorders. This is due, in part, to the increases in circulating remnant lipoproteins after TG have been transported for storage or hydrolysis. Raised TG, are also associated with an atherogenic lipoprotein phenotype (High TG, low high-density lipoprotein cholesterol (HDL-c) and small, dense low-density lipoprotein (LDL) particles). In addition, elevated TG contribute to ectopic storage of fatty acids in liver, adipose and muscle tissues, contributing to insulin resistance in all three tissues and consequent metabolic dysregulation. It is therefore paramount to prevent frequent and prolonged exposure to raised TG in the postprandial period, particularly in groups who are at increased risk of cardio-metabolic disease.

The dyslipidaemic component of cardio-metabolic health can be inferred by assessing the capacity to breakdown and clear TG from circulation after high fat ingestion using an oral fat tolerance test (OFTT). The OFTT can also be used to assess the efficacy of interventions targeting reductions in postprandial TG. Exercise and nutritional interventions have been shown to alter postprandial TG excursions and provide insight in to underlying mechanisms of postprandial lipid metabolism. However, there are several topics within this area of research that require further clarification. These topics have been addressed within this thesis.

The first experimental chapter of this thesis (chapter 3) aimed to investigate the repeatability of an OFTT (75g fat, 22g carbohydrate, 14g protein) designed to meet recommendations from an expert panel statement. This study also aimed to evaluate the repeatability of the postprandial response to an OFTT preceded by 1 hour of acute moderate intensity exercise (cycling at a work rate eliciting 90% oxygen consumption anaerobic threshold). After an overnight fast, 11 healthy adult male participants

consumed OFTT meals on 4 separate occasions; 2 preceded by rest and 2 preceded by exercise. TG area under the curve (AUC) was calculated for each test and compared to the repeat condition using non-parametric Bland-Altman analysis. The 4-hour OFTT was repeatable in the rest condition, with 9 of 10 repeat measurements falling within  $\pm 15\%$  of the median TG AUC (predefined as the upper limit of acceptable error). However, in the exercise condition repeatability was poor with only 2 of 11 repeat measurements falling within 15% of the median TG AUC.

Adult offspring of type 2 diabetics (OT2D) show irregular TG responses to OFTT with high or low carbohydrate content, compared to healthy controls. Prior acute aerobic exercise may favourably influence these postprandial responses in OT2D. This feasibility study (Chapter 4) aimed to investigate the effects of carbohydrate content and acute exercise on TG AUC after OFTT in OT2D. On 4 separate days, 8 adult male OT2D ingested OFTTs with low (HFLC; 75g fat, 22g carbohydrate, 14g protein) or high (HFHC; 75g fat, 95g carbohydrate, 14 g protein) carbohydrate content. Participants rested or exercised (1-hour moderate intensity; 90% oxygen consumption at anaerobic threshold) the day before each OFTT. Recruitment to the single centre was slow, but participant adherence to the study was good. There were large effect sizes for lower TG AUC and incremental AUC (iAUC) in the HFHC with prior exercise. Insulin AUC was higher in HFHC conditions and there was a large effect size for lower insulin AUC in the exercise conditions. Given the large effect sizes observed for the effects of prior acute exercise on postprandial TGs, an adequately powered multi-centre study was deemed to be relevant and feasible.

Consumption of strawberries appears to be beneficial in attenuating the postprandial lipaemic response to OFTT due to the high polyphenol content within strawberries. The mechanisms of this attenuation in postprandial lipaemia appear to be different from the

mechanisms involved in exercise induced reductions in postprandial lipaemia. However, the combined effects of exercise and strawberry interventions in reducing postprandial lipaemic responses to OFTT has not been investigated. The final experimental chapter (Chapter 5) aimed to evaluate the combined effects of acute exercise and strawberry consumption on postprandial responses to OFTT (73g to 74g fat, 32g to 33g carbohydrate, 11g to 12g protein). On 4 separate days, ten overweight/obese males ingested OFTTs with 25g freeze dried strawberries or a placebo. Participants rested or exercised (40 minutes submaximal high intensity exercise, HIIE) the day before each OFTT. There was a 20% reduction in TG AUC in the exercise conditions and no differences in TG AUC in the strawberry conditions.

This thesis offers key contributions to postprandial lipid metabolism research. First, the OFTT recommended by an expert panel statement is repeatable. Second, the variability observed in postprandial responses to OFTT with immediate prior exercise may explain the inconsistencies within the literature. Third, acute exercise showed a substantive effect in reducing TG AUC and iAUC with high carbohydrate OFTT in adult OT2D. These improvements could be explained by acute improvements in insulin sensitivity, however, a further adequately powered study is required to support the findings of this feasibility study. Finally, acute HIIE appears to be an effective strategy to reduce postprandial TG, but strawberry intake does not appear to improve postprandial TG.

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## Peer reviewed published articles and conference presentations

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## List of Abbreviations

Apo; Apolipoprotein	HIIE; High intensity interval exercise
AT; Anaerobic threshold	HMG CoA; 3-hydroxy-3-methyl-glutaryl-coenzyme A
AUC; Area under the curve	HRR; Heart rate reserve
BMI; Body mass index	iAUC; Incremental area under the curve
CHO; Carbohydrate	ICAM1; Intracellular adhesion molecule 1
CM; Chylomicron	IDL; Intermediate density lipoprotein
cm; Centimetre	IL; Interleukin
CO <sub>2</sub> ; Carbon dioxide	kcal; Kilocalorie
CPET; Cardiopulmonary exercise test	kg; Kilogram
CRP; C-reactive protein	l; Litre
CVD; Cardiovascular disease	li; Low intensity
Da; Daltons	LDL; Low density lipoprotein
DNL; De novo lipogenesis	LDL-c; Low density lipoprotein cholesterol
EDTA; Ethylenediaminetetraacetic acid	LGI; Low glycaemic index
EPOC; Excess post oxygen consumption	LOA; Limits of agreement
F; Female participant	LPL; Lipoprotein lipase
FOX; Ferrous oxidation-xylenol orange	m; Metre
g; Grams	M; Male participant
h; Hour	Mg; Milligram
HDL; High density lipoprotein	min; Minute
HDL-c; High density lipoprotein cholesterol	ml; Millimetre
HFHC; High fat high carbohydrate	mmHg; Millimetres of Mercury
HFLC; High fat low carbohydrate	mmol; Millimole
HGI; High glycaemic index	mod; Moderate intensity exercise
hi; High intensity	MRS; Magnetic resonance spectroscopy

n; Number of participants	TG; Triglyceride
O <sub>2</sub> ; Oxygen	TNF $\alpha$ ; Tumour necrosis factor alpha
NEFA; Non-esterified fatty acid	TRL; Triglyceride rich lipoprotein
NHS; National Health Service	VCAM1; Vascular cell adhesion molecule 1
nm; Nanometre	VLDL; Very low density lipoprotein
NR; Not reported	$\dot{V}O_2$ ; Rate of oxygen uptake
OFTT; Oral fat tolerance test	$\dot{V}O_{2max}$ ; Maximum oxygen uptake
OGTT; Oral glucose tolerance test	$\dot{V}O_{2peak}$ ; Peak oxygen uptake
OT2D; Adult offspring of type 2 diabetes	$\dot{V}O_{2R}$ ; Oxygen uptake reserve
oxLDL; Oxidised low density lipoprotein	VT1; Ventilatory threshold 1
PL; Phospholipid	VT2; Ventilatory threshold 2
Q; Quartile	W; Watt
s; Second	95%CI; 95% confidence intervals
SD; Standard deviation	$^{\circ}C$ ; Degrees Celsius
	$\eta p^2$ ; Partial eta squared

# Chapter 1: General Introduction

## 1.1 Overview

Cardio-metabolic disorders increase the risk of developing type 2 diabetes (T2D) and cardiovascular disease (CVD) and include obesity, hypertension, dyslipidaemia, raised blood glucose and insulin resistance (Alberti et al., 2009). An individual presenting with three or more CVD risk factors are considered to have a condition termed the metabolic syndrome. The metabolic syndrome affects an estimated 20-25% of the Western adult population (O'Neill and O'Driscoll, 2015). The metabolic syndrome is associated with a 1.6 fold increase in all-cause mortality risk (O'Neill and O'Driscoll, 2015) and increases the likelihood of developing T2D and CVD (Alberti et al., 2009). T2D is independently associated with development of CVD, with 80% of deaths in people with T2D attributed to CVD (International Diabetes Federation, 2015). CVD is the most frequent cause of death worldwide, claiming approximately 17.3 million lives each year (Townsend et al., 2016). Treatment of cardio-metabolic disorders and the metabolic syndrome is prudent because development of T2D and CVD is associated with increased risks of morbidity and mortality, decreased quality of life, and increased financial/workforce burden on health care services (World Health Organisation, 2005).

A common underlying feature of cardio-metabolic related disease progression is the dysfunctional handling of lipids after dietary intake and/or endogenous lipid production (Boren et al., 2014). Dysfunctional lipid metabolism appears to be central to the pathogenesis of skeletal muscle, hepatic and adipose tissue insulin resistance, a major risk factor for cardio-metabolic disease progression (Savage et al., 2007). Consequently, there are clearly defined treatment strategies targeting modifications in lipids and lipoproteins associated with cardio-metabolic disease progression. Treatment strategies



include lifestyle interventions (dietary intervention and increased physical activity/exercise), and drug therapies, such as statin and other hypolipidaemic medications (Catapano et al., 2016). Traditionally lipids are measured by analysing fasting blood samples. However, more recently, the postprandial period has become recognised as important for the assessment of metabolic health (Catapano et al., 2016). Following fat consumption, circulating lipids are elevated for 6-8 hours and westernised dietary habits typically include 3-4 meals per day. Accordingly, most of the day is spent in the postprandial state. Postprandial measures may therefore better reflect cardio-metabolic health compared to fasting measures (Lopez-Miranda et al., 2007).

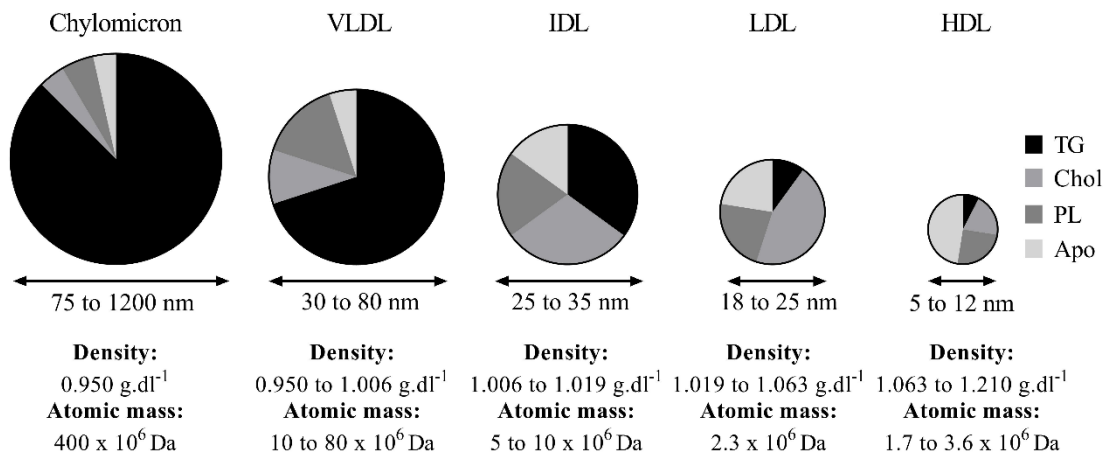
Postprandial lipid metabolism can be assessed by using specifically designed high fat meal challenges, commonly termed oral fat tolerance tests (OFTTs). These evaluate the capacity of the metabolic system to acutely process high levels of dietary fat in a defined period of time, often between 4 and 8 hours (Kolovou et al., 2011, Lairon et al., 2007, Maraki et al., 2011). Elevated plasma/serum concentrations of triglycerides (TG) in the postprandial state are associated with cardio-metabolic dysregulation (Nordestgaard and Varbo, 2014). Interestingly, postprandial cardio-metabolic dysregulation can be improved with acute exercise and may be influenced by nutritional factors such as the carbohydrate content (Kriketos et al., 2003) of the OFTT and high polyphenol fruit supplementation (Burton-Freeman et al., 2010). However, optimal interventions to reduce postprandial dysregulation, particularly in those at risk of cardio-metabolic disease require, further investigation. This chapter will introduce key literature related to;

- Fasting lipids and non-fasting lipids in assessing cardio-metabolic risk
- Risk factors that increase cardio-metabolic risk such as obesity and family history of T2D

- The role of the oral fat tolerance test and its scientific validity for assessment of dysfunctional metabolism
- Exercise as a therapeutic strategy for impaired postprandial lipid metabolism
- The potential for polyphenol supplementation to improve postprandial lipid metabolism

## 1.2 Lipids and related cardio-metabolic diseases

In the human body, lipids are stored within adipose, muscle and hepatic tissues and are transported in the forms of cholesterol and TGs, contained within lipoproteins in blood circulation. Lipoproteins (proteins containing lipids) are classified according to their size, density, carrier proteins and content of cholesterol and TGs (Ginsberg et al., 2005). The main classes of lipoproteins within the circulation are chylomicrons (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL) and high density lipoprotein (HDL) (*Figure 1.1*). These range in size and density with HDL being the smallest and CM being the largest particles (Nordestgaard and Freiberg, 2011). Within these classes of lipoproteins, ultracentrifugation can be used to define lipoprotein subclasses. These subclasses are determined by their size according to their Svedberg Flotation rate (Sf). For example, VLDL is often separated into 2 subclasses; large VLDL1 (Sf, 60 to 400) and smaller VLDL2 (Sf, 20 to 60), with VLDL1 being associated with greater cardiovascular risk (Colhoun et al., 2002, Gill et al., 2004). After the TG within lipoproteins are transported and deposited within tissues, LDL and TG deplete CM and VLDL remnants are transported to the liver. However, these particles may infiltrate arterial walls causing inflammation and oxidative stress, considered by some to be a causal process in atherogenesis (Nordestgaard, 2016). Conversely, HDLs remove cholesterol from tissues via direct and indirect pathways, one of these direct pathways facilitates the removal of circulating TGs (Rader and Hovingh, 2014). Accordingly, raised HDL concentrations are inversely related to cardiovascular events (Tuteja and Rader, 2014). Therefore the circulating concentrations and the composition/functionality of lipoproteins are an important marker of poor cardio-metabolic health (Catapano et al., 2016). This section will provide a brief overview of the lipoproteins that are central to cardio-metabolic health with a particular focus on those that are acutely altered with dietary intake of fats.



**Figure 1.1** Lipoprotein classes; size (nanometre, nm), density (g.dl<sup>-1</sup>), mass (Daltons, Da) and composition (%). Components are; Triglycerides (TG), Cholesterol (Chol), Phospholipid (PL), Apolipoproteins (Apo). Adapted from data presented by Ginsberg and colleagues (2005).

### 1.2.1 Cholesterol

Cholesterol is a type of lipid, in the sterol family, and is essential for cell structure and function and in the formation of bile salts and steroids (such as adrenaline) in humans. However, raised circulating total and low-density lipoprotein cholesterol concentration are risk factors for CVD (Catapano et al., 2016). The processes involved with CVD progression are complex and dyslipidaemia is one of the key contributing factors (Ginsberg and Fisher, 2009). Subsequently, the measurement of total cholesterol and LDL-cholesterol (LDL-c) to evaluate CVD risk is widely advocated (Catapano et al., 2016). Circulating cholesterol concentrations are fairly stable throughout the day and therefore can be measured with or without fasting to evaluate health status (Peddie et al., 2012). A total circulating cholesterol level  $\geq 7.5$  mmol.l<sup>-1</sup>, independent of other cardio-metabolic risk factors, requires treatment/intervention (Conroy et al., 2003, Catapano et al., 2016). Lower cholesterol levels require consideration of other risk

factors such as age, gender, blood pressure and smoking history (Conroy et al., 2003), in addition to other lifestyle factors and co-morbidities including social deprivation, psychosocial stress and chronic kidney disease (Catapano et al., 2016). Target LDL-c and HDL-cholesterol (HDL-c) concentrations for people with low to moderate risk of CVD are  $<3.0 \text{ mmol}\cdot\text{l}^{-1}$  (men and women) and  $>1.0 \text{ mmol}\cdot\text{l}^{-1}$  (men) or  $>1.2 \text{ mmol}\cdot\text{l}^{-1}$  (women), respectively (Catapano et al., 2016). The prognostic capacity of total cholesterol is improved when the size and density of its constituents are evaluated (Nordestgaard, 2016). High concentrations of circulating LDL-c and low concentrations of HDL-c are a feature of dyslipidaemia and a risk factor for CVD (Ginsberg, 2000). There are various causes of increased total cholesterol and LDL-c such as obesity, physical inactivity, poor dietary intake and an inherited predisposition based on numerous genetic abnormalities identified in pathways of lipoprotein metabolism (Nelson, 2013, Warburton et al., 2006). In most cases cholesterol can be maintained within target concentrations by; 1. A balanced dietary intake of fat, carbohydrate, protein, vitamins and minerals from good quality sources with total calorie intake similar to daily expenditure (Mozaffarian, 2016), and 2. Participating in regular physical activity (3-5 times per week and total physical activity  $>150$  minutes per week (Pescatello and American College of Sports Medicine, 2014)). Failure to meet these requirements or having a predisposition to dyslipidaemia increases the likelihood of dyslipidaemia and subsequent insulin resistance and cardio-metabolic disease progression.

### 1.2.2 Triglycerides

A TG is composed of one glycerol and three fatty acids. The primary role of the TG is to transfer energy to tissues for utilisation or storage and adipose tissue for storage

(Ginsberg and Fisher, 2009). TGs are transported within complex structures known as TG rich lipoproteins (TRL). TRL consist of CM (produced within the intestine) and VLDL (produced within the liver), and their remnants. A feature of dyslipidaemia is hypertriglyceridaemia, an excess in circulating TGs in the fasting and/or non-fasting state (Catapano et al., 2016, Srikanth and Deedwania, 2016). Hypertriglyceridaemia is caused by a dysregulation in the synthesis, hydrolysis and/or catabolism of TRL (Ginsberg, 2000, Boren et al., 2014). Such dysregulation may be genetically inherited and is strongly associated with obesity, type 2 diabetes (T2D) and development of CVD (Ginsberg, 2000). Unlike cholesterol, there are considerable increases in postprandial TG levels after consumption of dietary fats. Fasting and postprandial TGs are therefore independent markers of dyslipidaemia and independent predictors of CVD (Nordestgaard et al., 2007).

### 1.2.3 Elevated triglycerides and cardio-metabolic disease

The relationship between elevated TGs and obesity with coronary artery disease has been known for more than 35 years (Albrink et al., 1980, Zilversmit, 1979). However, as highlighted by Nordestgaard (2016), for the majority of this time, increased LDL-c and decreased HDL-c (and not TGs) were thought to be key mechanisms of CVD progression and were therefore primary treatment targets. Raised LDL-c has been consistently shown to be strongly associated with increased CVD risk and reducing LDL-c, through intervention (primarily 3-hydroxy-3-methylglutaryl-coenzyme A [HMG CoA] reductase inhibitors), is strongly associated with decreased CVD risk (Nordestgaard, 2016, Collins et al., 2016, Fulcher et al., 2015). HMG CoA reductase inhibitors compete with HMG CoA reductase in the liver and alter the conformation of this enzyme to prevent conversion of HMG CoA in to mevalonic acid, a precursor to

LDL-c (Stancu and Sima, 2001). Reduced hepatic LDL-c synthesis leads to increased clearance from circulation, subsequently reducing cardiovascular risk (Stancu and Sima, 2001). The strong associations between low HDL-c and CVD were assumed to be causal (Nordestgaard and Freiberg, 2011). However, in studies where medication successfully increased HDL concentrations, CVD progression was not attenuated (Nordestgaard, 2016, Stauffer et al., 2013, Briel et al., 2009). A recent meta-regression analysis of approximately 300 000 participants at risk of CVD across 108 randomised intervention trials reported that increased HDL-c levels, through drug intervention, had no effect on cardiovascular outcomes (Briel et al., 2009). There was no association between HDL-c and coronary heart disease related events, mortality or all-cause mortality when data were adjusted for LDL-c concentrations. Notably, change in HDL-c explained very limited variability (<1%) in these outcome measures. Furthermore, another recent meta-regression analyses of 40 randomised controlled trials that included 200 000 participants demonstrated that medication related reductions in TGs and/or LDL-c reduced cardiovascular events but medication related increases in HDL-c had no effect on reducing cardiovascular events (Stauffer et al., 2013). It is now apparent that increased TGs and reduced HDL-c are tightly co-regulated and that low HDL-c reflects prolonged exposure to hypertriglyceridaemia (Nordestgaard and Varbo, 2014). As such, recent attention has returned to investigate the role of raised TGs and their contribution to mechanisms of CVD progression (Nordestgaard, 2016).

TGs measured in either the fasted or non-fasted state are associated with development of cardio-metabolic disease (Austin et al., 1998, Bansal et al., 2007, Boullart et al., 2012, Ginsberg, 1997, Hyson et al., 2003, Jackson et al., 2012, Lewis and Steiner, 1996, Pirillo et al., 2014). A recent scientific consensus statement from the American Heart Association confirmed that TGs are an important biomarker of CVD risk, particularly due to their association with atherogenic remnant particles (Miller et al., 2011). To

support the prognostic importance of hypertriglyceridaemia, a large meta-analysis, of 29 studies, including 262 000 participants with approximately 10 000 cases of coronary heart disease demonstrated that raised TGs have a moderately strong association with coronary heart disease risk after controlling for HDL concentrations (Sarwar et al., 2007). In addition, in a 14 year follow-up study of 151 patients at a single preventative cardiology centre, when LDL-c was reduced with statin therapy, raised TGs were independently associated with increased CVD risk (Miller et al., 2007). Furthermore, in a study of 60 600 participants with 10 600 patients diagnosed with ischaemic heart disease, low grade inflammation (using participant CRP concentrations) was found to be a feature central to cardio-metabolic disease and appears to be related to hypertriglyceridaemia and remnant cholesterol but not raised LDL-c concentrations (Varbo et al., 2013b). This highlights the importance of raised TG independent of other measures (Varbo et al., 2013b). In a meta-analysis of 62 000 patients enrolled on 8 randomised controlled trials investigating the effects of statins on circulating lipids, the capacity for LDL-c to predict major cardiovascular events was significantly improved when non-HDL-c, which includes TG, was considered (Boekholdt et al., 2012). Finally, in a study of 73 500 patients with almost 12 000 diagnosed with ischaemic heart disease, increased exposure to TG rich remnants was found to be an independent risk factor for development of CVD (Varbo et al., 2013a). These large scale epidemiology and meta-analysis studies all indicate that elevated TGs are an important risk factor in cardio-metabolic disease.

In order to reduce fasting and postprandial TG concentrations, lifestyle interventions that are related to lower circulating TG concentrations and improved cardio-metabolic health are recommended. These include; reducing bodyweight, reducing carbohydrate intake, reducing trans-fat and saturated fat intake, and increasing physical activity (Miller et al., 2011). The effects of acute dietary and exercise interventions to lower



postprandial TGs will be appraised later in this chapter. First, the mechanisms of fat metabolism, postprandial dysregulation and methods used to assess postprandial lipid metabolism will be considered.

#### 1.2.4 Metabolism of exogenous triglycerides

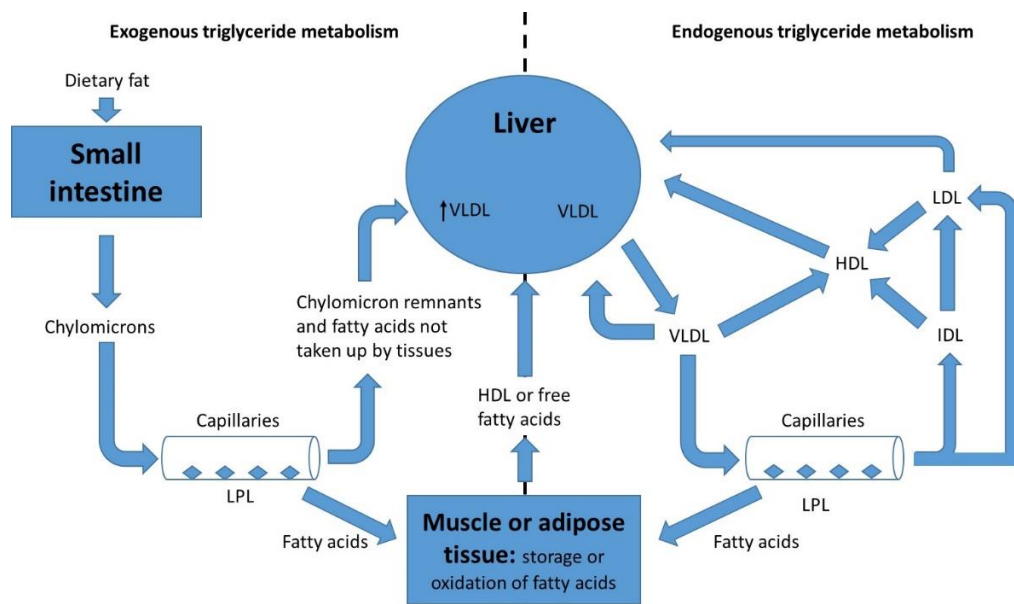
After eating fats, TGs (derived from these fats) enter the circulation after approximately one hour (Xiao and Lewis, 2012). The majority of the longer chain fatty acids are packaged within CM and enter the blood stream via the lymph system (Xiao and Lewis, 2012). A number of processes occur to breakdown complex fat structures from their food form to the TGs contained within CM. The breakdown of fats is initiated in the mouth by secretion of lingual lipases and then in the stomach with the addition of gastric enzymes (Iqbal and Hussain, 2009). Further emulsification and hydrolysis occurs as the fats pass through the duodenum (Iqbal and Hussain, 2009). TGs are hydrolysed within the jejunum in to glycerol and free fatty acids and are transported to enterocytes (Iqbal and Hussain, 2009). Free fatty acids and glycerol are then synthesized in to TGs within the endoplasmic reticulum by fatty acid binding proteins (produced by either the liver or intestine) in reactions that are catalysed by monoacylglycerol acyltransferase or diacylglycerol transferase enzymes (Iqbal and Hussain, 2009, Julve et al., 2016). TGs are then bound to proteins to form a CM, one of the key proteins that binds to the TGs form the CM is Apolipoprotein (Apo) B48 which is only produced by the gut (Julve et al., 2016). After fat consumption, the production of the CM particles within the gut is not increased, instead CMs increase vastly in size (Hayashi et al., 1990). TG rich CMs, consisting predominantly of TGs and monoglycerides (85-92%) (Iqbal and Hussain, 2009), are then released in to the circulation where they attach to other key apoproteins including; Apo CII, Apo CIII and Apo E. Apo CII facilitates

activation of lipoprotein lipase (LPL) for CM removal from circulation (Julve et al., 2016). Contrarily, Apo CIII inhibits LPL activity (Julve et al., 2016). Once in the circulation, CMs have an approximate half-life of 5 minutes and are hydrolysed by LPL, an enzyme located on endothelial cells and within adipocytes, skeletal and cardiac muscle cells (Karpe et al., 2007). CM has a greater affinity to LPL than VLDL, both of which compete for hydrolysis. This leads to a prolonged increase in circulating VLDL. On exposure to LPL, fatty acids are released from the CM either within the circulation where they are taken up by the liver, or are taken up by the adipose or muscle tissue for storage or oxidation (Ginsberg et al., 2005). The CM remnants are mostly removed by the liver which is facilitated by Apo E on the CM remnant binding to LDL receptors within the liver (Ginsberg et al., 2005). CM remnants can also be removed by entering subendothelial spaces within the vasculature, causing inflammation and contributing to the development of atherosclerosis (Nordestgaard and Varbo, 2014). This finding has been supported by identifying lipoprotein remnants within the arterial intima in animal studies (Proctor et al., 2000). After approximately 5-8 hours, provided that no further fats have been ingested, circulating CM returns to low circulating levels.

#### 1.2.5 Metabolism of endogenous triglycerides

VLDL is continuously secreted by the liver and is a major transporter of TGs (Karpe et al., 2007, Karpe, 1999). TGs can be delivered to, and synthesised within the liver through several pathways; TGs within VLDL and CM remnants are delivered to the liver, fatty acids are secreted by muscle or adipose tissue, TGs can also be synthesised endogenously via de novo lipogenesis (DNL) (Karpe, 1999). VLDLs can be differentiated from CMs by the apolipoprotein B100 attached to the TRL. Production of VLDL is increased when fatty acid or TG delivery to the liver is increased, and

production is inhibited (in healthy humans) when insulin concentrations are increased (Karpe et al., 2007). Interestingly, the size of VLDL may be influenced by exercise resulting in an increase in the affinity of VLDL for LPL (Ghafouri et al., 2015). Once in circulation, VLDL TGs can be removed after binding with LPL in a similar fashion to CMs. However, CMs have an estimated 50 fold greater affinity for LPL, as such VLDL concentrations often rise in the postprandial period (Karpe et al., 2007). VLDL that are hydrolysed by LPL may be reduced in size to form LDL or become VLDL remnants (IDL), depending on the degree of hydrolysis. HDL also removes TGs from VLDL in exchange for cholesterol ester transfer protein. The TG enriched HDL are more readily removed from circulation by liver hepatic lipase, resulting in fewer circulating HDL particles and thus a reduced cardio-protective capacity (Lamarche et al., 1999). The smaller IDL and LDL particles become enriched with cholesterol, increasing their atherogenic properties, and their smaller size may enable infiltration of endothelial walls (Zilversmit, 1979). However, most remnants will be hydrolysed by the liver LDL receptors (Lopez-Miranda et al., 2007). Exogenous and endogenous pathways of TG metabolism are presented in *Figure 1.2*.



**Figure 1.2** Endogenous and exogenous triglyceride metabolism. Adapted from Lopez-Miranda et al. (2007).

### 1.3 Postprandial lipaemia and cardio-metabolic disease

Postprandial lipaemia is a term used to describe the increase in circulating TG within TRL and their remnants during the hours after dietary fat ingestion (Lopez-Miranda et al., 2007). Unlike fasting conditions, where TGs and TRL remain fairly stable, the postprandial period involves a complex chain of events to metabolise or store fats (described earlier). These events can elicit the onset of vascular pathologies, particularly where TG concentrations are frequently increased for prolonged time periods (Lopez-Miranda et al., 2007). Age, gender, menopausal status, dietary habits, physical activity, smoking status, genetic inheritance, as well as metabolic pathologies such as obesity, insulin resistance and type 2 diabetes impact on the postprandial lipaemic response (Pirillo et al., 2014). Insulin plays an important role in controlling the secretion (Malmstrom et al., 1997, Malmstrom et al., 1998) and removal (Panarotto et al., 2002) of TRL, therefore risk of CVD related pathologies are increased in insulin resistant conditions with hyperinsulinaemia. In those with cardio-metabolic disorders, after consuming a standardised amount of fat, TG levels can remain elevated for twice as long and reach peaks 2-3 times greater compared to healthy controls (Cohn, 2006). A leading hypothesis for the mechanism underlying this increase in postprandial TG has been demonstrated in well-designed studies by Rabøl and colleagues (2011) and Petersen and colleagues (2007). First, Petersen and colleagues (2007) demonstrated that muscle insulin resistant adult offspring of people living with type 2 diabetes (OT2D) had a two-fold rise in postprandial TG after a high carbohydrate mixed meal compared to healthy controls. Using Magnetic Resonance Spectroscopy (detailed later in section 1.3.2.2) they identified that this was due to increased de novo lipogenesis (DNL) (60%) and TG synthesis, reduced HDL (20%) and a decreased rate of clearance of TG and glucose at the muscle (Petersen et al., 2007). This hypothesis was further supported by evaluating responses to the same high carbohydrate mixed meals, using exercise on one

of the study days to acutely improve insulin sensitivity in insulin resistant lean males. Exercise (3x 15 minutes at 75 to 85% calculated maximum heart rate) increased glycogen synthesis, and decreased hepatic TG synthesis and DNL (Rabol et al., 2011). This study demonstrated that muscle insulin resistance can be acutely reversed and that this improves TG mediated clearance (Rabol et al., 2011). Identifying a larger and more prolonged exposure to TG-rich lipoproteins and their remnants (IDL, VLDL and CM remnants) is important because of the strongly associated increased pathologies associated with dyslipidaemia.

A causal role between postprandial TGs and atherosclerosis was proposed by Zilversmit in 1979 (Zilversmit, 1979). Many studies have been conducted since this proposal to better understand the pathophysiological processes involved. Postprandial lipaemia has since been associated with acute endothelial dysfunction (Vogel et al., 1997), impaired platelet function (Tamburrelli et al., 2012, Michelsen et al., 2009), systemic inflammation and oxidative stress (de Vries et al., 2014). Increases in circulating VLDLs and CMs are associated with development of atherosclerosis (Rapp et al., 1994, Karpe et al., 1994, Tomkin and Owens, 2001). After LPL hydrolysis, VLDLs and CMs become smaller and denser remnant particles which can infiltrate arterial walls (Pirillo et al., 2014). The arterial wall infiltration by these remnant particles, containing 5-20 fold more cholesterol than LDL particles, is likely causal in atherosclerosis (Wilhelm and Cooper, 2003). Potential mechanisms include inflammation, macrophage formation and endothelial dysfunction (Pirillo et al., 2014). However, more novel mechanisms have recently been identified and discussed by de Vries and colleagues (de Vries et al., 2014). It has become apparent that prior to the pathways of postprandial atherogenesis mentioned above, there is an inflammatory vascular response that is proposed to be activated by remnant lipoproteins binding with white blood cells (Alipour et al., 2008b). Incidentally, there is a greater content of lipids within the white blood cells of those

with CVD compared to apparently healthy people (Tertov et al., 1992). During postprandial fat metabolism, activation of neutrophils may contribute to the postprandial increase in the production of reactive oxygen species (ROS) that cause subsequent endothelial dysfunction (van Oostrom et al., 2003). Exposure of LDL to ROS leads to modification of the LDL in to oxidised LDL (oxLDL) (de Vries et al., 2014). CM remnants and oxLDL particles that penetrate the arterial walls, are taken up by macrophages and form foam cells, initiating the formation of an atherosclerotic plaque (Teeman et al., 2016). These processes contribute to the release of modulators of endothelial function, such as vascular cell adhesion molecule 1 (VCAM1) and inflammatory markers including interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor alpha (TNF $\alpha$ ) (de Vries et al., 2014). The inflammatory and oxidative stresses caused by these processes are hypothesised to cause systemic inflammation and endothelial dysfunction, both of which are prognostic in CVD (de Vries et al., 2014, Cai and Harrison, 2000, Halcox et al., 2002).

The lipid handling capacity of the body can be assessed by feeding a moderate or high amount of fat to a fasted individual and measuring postprandial circulating TG concentrations at predefined time points. This test has been termed the oral fat tolerance test (OFTT). At present, the OFTT is not widely used in a clinical setting for assessment of cardio-metabolic function. One reason for this could be the time demands of OFTT (Weiss et al., 2008) which typically require postprandial measurements to be taken every 1 or 2 hours for 5-8 hours (Zhang et al., 2004, Plaisance et al., 2008, Pfeiffer et al., 2005, Petridou et al., 2004, Katsanos and Moffatt, 2004, Clegg et al., 2007). The test is required to last several hours because TGs typically reach a peak at 3 to 4 hours after lipid ingestion and resolve to baseline concentrations at 6 to 8 hours (Pirillo et al., 2014). Recently, an abbreviated OFTT (lasting 4-hours) has been developed and validated (Weiss et al., 2008, Maraki et al., 2011). This test reduces time constraints and

improves the practicality of OFTT (Weiss et al., 2008). Furthermore, the 4-hour time point has been recommended by an expert consensus as clinically relevant and the most representative time to measure postprandial TG responses following an OFTT (Kolovou et al., 2011). However, the test re-test repeatability of the abbreviated 4-hour OFTT has only been investigated in one single centre study with a small population (Weiss et al., 2008). It is important to understand the test-retest repeatability to ascertain the typical error in the measurement. Understanding the measurement error enables identification of whether changes in repeat tests used for serial clinical assessment or acute interventions fall within or outside of the error in the measurement.

### 1.3.1 Oral fat tolerance test (OFTT) meal composition

#### *1.3.1.1 Fat quantities in OFTT*

The composition of the OFTT should be considered prior to experimental study or clinical use. This is because the amount of fat, the type of fat, and the presence of carbohydrate, fibre and protein may alter the postprandial lipaemic response (Lopez-Miranda et al., 2007). Five to 15 grams (g) of fat in a meal leads to insignificant increases in postprandial lipids (Dubois et al., 1998). Fat intakes of 30 to 50g within an OFTT, has been shown to increase TG concentrations in the region of 0.85 to 1.24 mmol.l<sup>-1</sup> above baseline values, in a dose dependent manner (Lopez-Miranda et al., 2007, Cohen et al., 1988, Murphy et al., 1995). This dose dependent relationship extends to approximately 80g fat. Meals containing fat above 80g causes an exaggerated lipaemic response which is not dose dependent (Plaisance and Fisher, 2014, Lopez-Miranda et al., 2007).

The amount of fat previously used in OFTT has been either a fixed quantity, or variable according to body mass or surface area (Lairon et al., 2007). Numerous methods have

been used in the literature to adjust fat meals to body mass, as can be observed in the reviewed exercise and postprandial lipaemia literature (Peddie et al., 2012). The main rationale for adjusting for body size/mass is to account for increases in blood volume in relation to body size. However, in those at greatest risk of cardio-metabolic dysfunction, the increased mass and surface area is likely to be caused by excess adiposity and therefore adjustments would likely overestimate blood volume in overweight/obese populations (Lairon et al., 2007). Furthermore, in order to adopt such a test in clinical practice, a fixed dose may be most appropriate to encourage standardisation among healthcare providers. The oral glucose tolerance test, which is widely adopted in clinical practice, takes this approach (WHO, 2006). An OFTT expert panel statement recently recommended to standardise fat doses at 75g (Kolovou et al., 2011). The use of a fixed fat content, rather than adjusting for body mass/size, has also been proposed to minimise errors in experimental studies particularly in homogenous populations (Lairon et al., 2007). A large meta-analysis recently reported that the optimal dose of fat in OFTTs is 70 to 79g (Mihas et al., 2011). This meta-analysis included 113 studies that evaluated postprandial responses to OFTTs in normolipidaemic, defined as fasting TG  $<2.0 \text{ mmol.l}^{-1}$ , non-obese (BMI  $< 30 \text{ kg.m}^{-2}$ ) Caucasian adults. Fat quantity was assessed between studies, with 10g increments used between 40g and 120g fat. The net change in circulating TG from baseline, termed the standardised mean difference, was highest with 70 to 79g fat. In addition, the authors indicated that 4 hours after fat ingestion identifies the most important time-point after fat ingestion due to TG reaching its highest circulating concentrations (Mihas et al., 2011). The authors concluded that the 4-hour time-point after 70 to 79g fat consumption was the most relevant for assessment of postprandial TG responses and identified a mean increase in TG of  $1.74 \text{ mmol.l}^{-1}$  from baseline to peak TG.



The 4-hour collection period has also been supported for use in CVD patients by a recent validation study evaluating the capacity of the OFTT to identify lipid dysmetabolism in a CVD cohort and in a general population cohort (Perez-Martinez et al., 2016). The postprandial TG responses from approximately 1000 participants with CVD and 1100 apparently healthy participants were retrospectively assessed. These data were taken from the CORDIOPREV (CVD cohort) and the GOLDN (healthy cohort) studies, where TG concentrations were assessed after high fat meal intake hourly for 4 hours or at 3.5 and 6 hours, respectively. An increase in TG in excess of 2.5 mmol.l<sup>-1</sup> was considered to be an abnormal response. The authors identified that 49% of CVD patients and 42% of apparently healthy participants with fasting TG between 1 and 2 mmol.l<sup>-1</sup> had abnormal responses to the OFTT. Of note, the authors reported that there was no additional benefit of OFTT over fasting measures in those who had fasting TG outside of the 1 to 2 mmol.l<sup>-1</sup> range.

#### *1.3.1.2 OFTT fatty acid content*

The type of fat used in OFTT may also be important. This topic has recently been systematically reviewed by Monfort-Pires and colleagues (Monfort-Pires et al., 2016). Eighteen studies were included, 12 of the studies compared saturated fat OFTT meals with polyunsaturated fat OFTT meals and 16 compared saturated fat OFTT meals with monounsaturated fat OFTT meals, 10 studies included both comparisons. Interestingly, there were no differences between saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids after 4 hours or 6 hours. However, at 8 hours, monounsaturated fatty acid and polyunsaturated fatty acid OFTTs provoked a lower lipaemic response compared to saturated fatty acids. The authors reported that a postprandial monitoring time of 4 hours was insufficient to differentiate TG responses to specific fatty acid types. The 8 hour TG response to a polyunsaturated fat OFTT was significantly lower than a saturated fat OFTT. There was also a trend for a lower TG

response after a monounsaturated fat OFTT compared with a saturated fat OFTT. These responses were not different at 4 hours. A recent study that compared the effects of a saturated fatty acid meal and a monounsaturated fatty acid meal on postprandial markers of acute cardiovascular and endothelial dysfunction also found no differences between the types of fatty acids (Lithander et al., 2013). However, the measures were taken at baseline and at 4 hours after meal ingestion. Given the importance of the 4-hour time period, and the similar responses at 4 hours between the different types of fats within the OFTT, in studies using fatty acids to provoke lipaemia and assess interventions to reduce this response, it may not matter which source of fatty acids are used.

#### *1.3.1.3 OFTT protein content*

A recent expert panel statement suggests that OFTT meals should contain 10g of protein (Kolovou et al., 2011). The rationale for this is related to the requirement of fat and protein within an OFTT meal to enable fat absorption (Kolovou et al., 2011). However, there is limited evidence on the influence of protein on postprandial lipaemic responses. Westphal and colleagues (2006) identified that two different types of protein (50g sodium caseinate or 50g soy protein) reduced the lipaemic responses to an OFTT containing an approximate mean fat content of 70g (Westphal et al., 2006). The authors reported that TG concentrations were lower at 1, 2 and 3 hour time-points after OFTT ingestion by approximately 20% (absolute numbers were not reported). It is important to note that insulin AUC values in the protein conditions were between 20 and 30% higher than the control condition. In addition, the extent of endothelial dysfunction, measured by flow-mediated dilation, from baseline (approx. 8.5%) was not as pronounced after the high protein OFTTs (trivial reductions) compared to the control OFTT (approximately 4% reduction) (Westphal et al., 2006). Milk and whey proteins may also improve postprandial insulin sensitivity (Nilsson et al., 2004, Akhavan et al., 2010). This may be important due to the regulatory role that insulin has over

upregulation of LPL activity and inhibition of VLDL secretion. Recently, postprandial lipaemia (TG iAUC) after an OFTT (80g fat, 45g carbohydrate, 45g protein) was reduced with whey protein compared to cod protein (61% higher than whey protein) and gluten (66% higher than whey protein) in obese non-diabetic participants (Holmer-Jensen et al., 2013). As such, the type of protein and probably the amount of protein should be considered when designing OFTT meals.

#### *1.3.1.4 OFTT carbohydrate content*

The carbohydrate content of OFTT meals recommended by an expert panel is 25g, with the rationale being that this amount enables adequate fat absorption (Kolovou et al., 2011). However, many studies evaluating responses to OFTTs have employed a much larger carbohydrate content (*Table 1.1*) (Gill et al., 2002, Burton-Freeman et al., 2010, Miyashita et al., 2006). In healthy participants, the lipaemic response is reduced if the glucose content of an OFTT is increased (Westphal et al., 2002, Kriketos et al., 2003). This could be related to delayed gastric emptying, shifting the postprandial TG curve rightwards. However, it may also be related to the hyperinsulinaemia that ensues after glucose ingestion (Kriketos et al., 2003). The regulatory effects of insulin on LPL and VLDL have already been described. Replacing glucose with fructose increases postprandial lipaemia (Chong et al., 2007). Fructose does not stimulate large increases in insulin secretion which may result in greater postprandial increases in TGs. Therefore, OFTT carbohydrate composition should be carefully considered. For OFTT experimental studies where the intervention may alter insulin sensitivity (such as an exercise intervention (Rabøl et al., 2011)), the carbohydrate content of the OFTT meal should be considered when evaluating the mechanism of TG clearance. Assessing responses to low and high carbohydrate OFTT meals may also provide further understanding of abnormal metabolic control in specific populations (Kriketos et al., 2005). This will be discussed later.

### 1.3.2 Measurements and reporting of postprandial lipid responses to an oral fat tolerance test (OFTT)

#### 1.3.2.1 *Triglyceride AUC and iAUC*

Due to the strong association between raised postprandial TG and CVD (Pirillo et al., 2014), plasma or serum TGs are commonly measured to describe responses to the OFTT. These measurements are often taken at baseline and every 1 or 2 hours for a total of 4 to 8 hours after meal ingestion. To evaluate the total TG response, these data are commonly presented as total area under the curve (AUC) (which includes the baseline measurement) or incremental AUC (where the baseline measurement is considered to be zero and the increase in TG from baseline is assessed) (Carstensen et al., 2003). Both AUC and iAUC are calculated using the trapezoidal method (Matthews et al., 1990).

TG AUC is more commonly reported in the postprandial lipaemia literature; a recent meta-analysis identified 121 TG AUC effect sizes reported in 76 randomised controlled trials compared to 70 effect sizes for TG iAUC (Freese et al., 2014). Using data from 3 previous studies, Carstensen and colleagues (2003) compared postprandial responses to OFTT (80g fat, 50g carbohydrate) in young (mean  $\pm$ SD age; 23  $\pm$ 2 years) apparently healthy (n= 10) and older (mean  $\pm$ SD age; 65  $\pm$ 5 years) T2D participants (n= 47). They concluded that because AUC correlated strongly with fasting TGs and iAUC correlated strongly with the rise in TG; iAUC better explains the postprandial lipid response (Carstensen et al., 2003). However, intervention studies, such as those employing acute exercise, lower both fasting and postprandial TG concentrations (Magkos et al., 2008b, Tsekouras et al., 2007). It could therefore be argued that AUC is more appropriate in intervention studies seeking to ameliorate excursions in circulating TG concentrations.

#### 1.3.2.2 *Tracing lipids and magnetic resonance spectroscopy (MRS)*

More complex analysis methods of assessing postprandial TG metabolism exist, such as magnetic resonance spectroscopy (MRS). Natural stable isotopes such as  $^1\text{H}$  and  $^{13}\text{C}$  can

be injected in solution or orally ingested in food with fats or carbohydrates containing these isotopes. These isotopes have the same chemical properties but a different atomic mass compared to the more commonly occurring isotope element (Magkos and Mittendorfer, 2009). This different mass enables its detection using either non-invasive techniques such as MRS (Hwang and Choi, 2015), or invasive blood sampling with the stable isotopes identified in plasma or serum by mass spectrometry (Magkos and Mittendorfer, 2009).

MRS offers a unique non-invasive in vivo method to evaluate underlying mechanisms of postprandial lipid metabolism, with the opportunity to assess specific tissues such as muscle and adipose tissue, and whole organs such as the liver (Hwang and Choi, 2015). A strong magnetic field is created by a super conducting magnet which allows identification of the chemical content of magnetic resonance visible nuclei (Blüml, 2013, Befroy and Shulman, 2011). Therefore the capabilities of this measurement provide biochemical and physiological information that would be difficult to assess through other measurement and imaging techniques (Hwang and Choi, 2015). The limitations of this technique include the expense, the requirement of experts to understand and process the data collected, the length of time required for scans and the lack of standardised protocols (Hwang and Choi, 2015). However, this appears to be an excellent research tool that is still in early development.

Tracers can also be measured within plasma and serum to investigate postprandial clearance of lipids (Lambert and Parks, 2012). A mass spectrometer is required for measurement of the natural isotopes present in serum or plasma. With recent advances in gas chromatography mass spectroscopy, this method has a higher sensitivity for lipids than liquid chromatography mass spectroscopy and therefore a smaller amount of tracer can be used in each dose (Lambert and Parks, 2012). These techniques can contribute to

TG measurements to aid understanding of underlying mechanisms of postprandial responses that cannot be achieved with serum or plasma analysis of circulating TG alone. Isotope tracers in addition to lipid quantities can also be assessed using more invasive or higher risk procedures such as muscle biopsies or positron emission tomography (requiring radioactive isotopes) (Lambert and Parks, 2012).

### 1.3.3 The repeatability of the oral fat tolerance test (OFTT)

The repeatability of the TG response to OFTTs, lasting between 5 and 8 hours, is reported to be high in healthy normolipidaemic (Weiss et al., 2008, Brown et al., 1992, Gill et al., 2005, Ryan et al., 2013) and obese populations (Weiss et al., 2008). However, it is unclear whether this measure is more variable in women compared to men as two studies report conflicting data (Gill et al., 2005, Weiss et al., 2008). There is not currently a standardised protocol for an OFTT in clinical practice. However, an OFTT typically involves consumption of a meal with either a fixed high fat dose, normally 70 to 80g of fat (Kolovou et al., 2011), or an amount of fat relative to the body mass of the individual, such as 0.7 to 1.5g fat per kilogram body mass (Freese et al., 2014). Blood samples are commonly taken each hour for up to 6 to 8 hours after OFTT meal ingestion to measure TG responses (Maraki et al., 2011, Weiss et al., 2008). Due to the lengthy nature of this test, a shortened 4-hour sampling period has been validated and shown to be highly predictive of the 8 hour test for TG response (Maraki et al., 2011, Weiss et al., 2008). Weiss et al. (2008) first investigated the reproducibility of the 4-hour test using an 800 kcal meal (71% fat) by testing 9 participants (2 male) on 4 separate occasions. Maraki and colleagues (2011) further evaluated the accuracy of the abbreviated test by assessing responses of 72 (32 male) participants to a single OFTT. This study demonstrated that a shorter 4-hour time period was able to predict 8 hour TG

AUC response to OFTTs. The key test-retest repeatability studies are considered below, throughout this body of text, data are presented as mean  $\pm$ SD unless an alternative is stated;

Brown and colleagues (1992) recruited 10 (7 female) volunteers aged 45 to 64 years old to consume an OFTT on two separate occasions within 10 days. The OFTT contained 1374 calories, 50g carbohydrate, 130g fat and 11g protein per 2 square metres of body surface area. Blood samples were taken at 3.5 and 9 hours into the postprandial period. No food consumption or drinks containing carbohydrates were allowed, however, unusually caffeine intake was permitted during the 9 hour postprandial period. To compare the responses across the two OFTT testing days, intra-class correlations were performed between the measurements at 3.5 hours and 9 hours. At 3.5 hours the correlation coefficient between TG responses for the two OFTTs was 0.76, and this was 0.85 at 9 hours. Despite permitting caffeine intake, which may reduce postprandial TG absorption (Jarrar and Obeid, 2014), these findings demonstrated a moderate to good reproducibility in TG measures. However, the amount of fat administered in this study was above the threshold at which postprandial lipaemia is exaggerated and does not increase proportionally to dose (Plaisance and Fisher, 2014, Lopez-Miranda et al., 2007).

Gill and colleagues (2005) recruited 20 (13 female) participants whose fasting lipid and glucose levels were within a 'healthy range'. The 7 men were  $44 \pm 10$  years and had a body mass index (BMI) of  $27 \pm 2$  kg.m<sup>-2</sup>. The 13 women were  $26 \pm 6$  years old and had a BMI of  $23 \pm 4$  kg.m<sup>-2</sup>. The OFTT meal contained 1.2g fat, 1.2g carbohydrate, and 17 kcal per kilogram of body mass. Only water was allowed to be consumed during the postprandial period and participants abstained from physical activity for the 3 days prior to OFTT and alcohol on the day prior to OFTT. The women were tested once during the

follicular phase and once during the mid-luteal phase of their menstrual cycle. This was to determine whether the menstrual cycle influenced the variation in the results. Blood samples were measured for glucose, insulin and TG at baseline, 30 minutes and one hour and then each hour for 6 hours. Within subject coefficient of variation between the two OFTT testing days was 10% for men, however, for 11 women (2 were excluded due to progesterone concentrations outside of the normal range) it was much larger at 23%. The authors concluded that the menstrual influences postprandial TG responses.

Weiss and colleagues (2008) recruited 5 (4 female) lean and 4 (3 female) obese subjects aged  $23 \pm 1$  and  $36 \pm 4$  years, respectively (Weiss et al., 2008). BMI was  $21 \pm 1 \text{ kg.m}^{-2}$  in the lean group and  $40 \pm 1 \text{ kg.m}^{-2}$  in the obese group. Fasting insulin and LDL-c concentrations were higher and HDL-c was lower in the obese group. Participants were prescribed a weight maintaining diet for 3 days prior to each meal test and were advised to avoid caffeine, alcohol and strenuous exercise 24 hours before each OFTT. Participants were provided with a standardised meal to consume on the evening before the OFTT. The OFTT contained 35g of fat per metre squared body surface area. Blood samples were taken each hour for 8 hours. The coefficient of variation for whole group total TGs area under curve (AUC) was 8% (95% confidence intervals [95%CI] 6% and 10%). In the 5 lean participants it was 9% (95%CI: 6% and 13%) and in obese it was 6% (95%CI: 4% and 8%). However, the iAUC coefficient of variation was 21% (95%CI: 16% and 26%) for the whole group. This study demonstrated high reproducibility of the AUC but this was lower for the iAUC for the 8-hour postprandial TG responses to OFTTs. In order to validate a shorter postprandial monitoring period, the authors also evaluated the variation of the first 4 hours of this test. The coefficient of variation for the 4-hour AUC was also low, 7% (95%CI: 5% and 8%) but again this was higher for the 4-hour iAUC; 16% (95%CI: 12% and 20%). For both the 8-hour and 4-hour monitoring periods the TRLs had lower reproducibility, suggesting that total TGs



are more reproducible than TRLs after an OFTT. The authors attributed this to greater error in measurements of samples. There are more analytical stages involved in the analysis for TRLs compared to total TGs, introducing potential for increased technical error.

Ryan and colleagues (2013) recruited 51 (26 female) healthy participants (age,  $30 \pm 12$  years; BMI,  $25 \pm 3 \text{ kg.m}^{-2}$ ) to perform two OFTTs within approximately 25 days (Ryan et al., 2013). The meal contained 533 kcal 54g fat and 11g carbohydrate. The authors reported that there were no significant differences and low variability in TG responses between visits, using ANOVA and a bespoke within person variability calculation, respectively. There were 9 patients identified who differed in TG response to a greater extent than others. Further analyses identified genotypic differences in this group in the following 7 genes: APO A1, IL1a, IL1b, TLR4, TCF7L2, CCK1Rec, and STAT3. The phenotype of this group was; elevated fasting TGs and elevated area under the curve in comparison to the whole cohort. The authors suggested that variable responses to a fixed load of oral fat may be an independent risk factor that is associated with differing genotype and phenotype.

To summarise, based on the existing literature, the OFTT appears to be a highly repeatable test. However, in women it may be prudent to control for the menstrual cycle (Gill et al., 2005). The 4 studies that have investigated the repeatability of the test used meals with different fat and carbohydrate proportions. One study did not control for caffeine intake. Nevertheless, the oral fat tolerance test was shown to be repeatable. Interestingly one study identified that people with high variability in postprandial TG responses after repeat OFTT may be a population at higher risk (Ryan et al., 2013). Further studies are required to validate this. The statistical assessment of agreement employed across these studies may not be the most appropriate for assessing agreement,

as discussed by Bland and Altman (Bland and Altman, 1999). Of importance to the series of studies proposed in this thesis, there is only one study that has investigated the repeatability of the abbreviated 4-hour OFTT (Weiss et al., 2008). Whilst data presented in this single study showed a good level of reproducibility, it is important that these findings are corroborated at other centres. In addition, despite many studies investigating the effects of prior exercise on postprandial lipaemia, the repeatability of the postprandial TG response to an OFTT preceded by exercise is unknown.

#### 1.4 Prior exercise and postprandial lipaemia

Physical activity encompasses all movements performed as part of daily living. Exercise is a component of physical activity involving a structured planned activity often with the aim to maintain or improve health and fitness. It is well established that increased physical activity lowers risk of CVD (Li and Siegrist, 2012, Berlin and Colditz, 1990), T2D (Colberg et al., 2010) and dyslipidaemia (Sui et al., 2017). In order to assess the specific beneficial effects of physical activity, exercise is often used as the mode of physical activity because it can be measured more easily and to a higher degree of accuracy within a laboratory setting.

The postprandial lipaemic response is lower in those who perform regular exercise compared to those who are sedentary (Tsetsonis et al., 1997, Herd et al., 2000). However, in a cohort of trained adult males and females who adopted sedentary behaviour for 60 hours, there were no differences in TG responses between this group and a lean comparator untrained (but reasonably fit, mean  $\dot{V}O_2\text{max}$ ; male  $51 \pm 5 \text{ ml.kg}^{-1}.\text{min}^{-1}$ , female  $39 \pm 4 \text{ ml.kg}^{-1}.\text{min}^{-1}$ ) group (Herd et al., 2000). A separate study in healthy men showed that the acute effects of exercise on reducing the postprandial response were diminished by 24 hours (Zhang et al., 2004). Therefore the benefits of

exercise in providing protective responses to postprandial lipaemia appear to be transient (Peddie et al., 2012) and only appear to last 24 hours in men (Zhang et al., 2004). Current physical activity recommendations suggest that at least 150 minutes of moderate intensity physical activity is performed 3-5 times per week (Pescatello and American College of Sports Medicine, 2014). Therefore, this may not be optimal for protection against postprandial lipaemia and its associated cardio-metabolic risk in those who do not meet and those at the lower end of the recommended range of physical activity levels.

The consistent effects of exercise in acutely reducing postprandial lipaemia is an area of research that has been comprehensively studied and reviewed in recent years (Freese et al., 2014, Maraki and Sidossis, 2013, Plaisance and Fisher, 2014, Burns et al., 2015, Teeman et al., 2016, Peddie et al., 2012). Studies that have been published since these reviews are described in *Table 1.1* and the fasting and postprandial lipid responses are reported in *Table 1.2*. These studies further substantiate the beneficial effects of prior exercise on postprandial lipaemia. Twelve of the 15 recently published studies report positive effects of prior exercise on reducing postprandial lipaemia. Most of the studies were conducted using young male populations and contained small sample sizes ranging from 6 to 29 participants. Two of the 15 studies investigated the effects of prior exercise  $\leq 2$  hours before an OFTT (Canale et al., 2014, Littlefield et al., 2017). Consistent with the mixed findings of previous studies investigating the effects of exercise performed within 4 hours of OFTT, reported in the recent review articles (cited above), one study showed a positive effect (Littlefield et al., 2017) and one showed no effect of immediate prior exercise on postprandial lipaemia (Canale et al., 2014). The influence that the timing of exercise before an OFTT has on postprandial TG responses is described in more detail later in this chapter.

The modality of exercise employed in the studies reported in *Table 1.1* was walking or running on a treadmill (9 studies) and cycling exercise on a cycle ergometer (6 studies). The duration of the exercise interventions ranged between 18 minutes (Freese et al., 2015) and 90 minutes (Kaviani et al., 2016). Studies that employed shorter duration acute exercise interventions often exercised participants at a higher intensity compared to studies that employed longer duration exercise interventions, particularly if two exercise intensities were being compared and energy expenditure was matched (Littlefield et al., 2017, Lopes Kruger et al., 2016). The exercise intensities in these studies ranged from walking at 50%  $\dot{V}O_{2peak}$  (Arjunan et al., 2015) to sprint cycling at 200% of peak power (Canale et al., 2014). Energy expenditure during exercise, where reported, ranged from 300 kcal (Lopes Kruger et al., 2016) to 930 kcal (Arjunan et al., 2013). Interestingly, of the two studies that investigated the effects of energy expenditure of approximately 300 kcal, one study showed that both moderate and high intensity exercise were effective in reducing postprandial lipaemia (Lopes Kruger et al., 2016), and the other study showed that moderate exercise did not significantly reduce postprandial lipids (Emerson et al., 2016). The meal composition also varied between trials, mean fat content for each study ranged from 52g (Lopes Kruger et al., 2016) to 100g (Littlefield et al., 2017). Despite a recent expert panel statement recommending 25g of carbohydrate (Kolovou et al., 2011), the carbohydrate content was greater than 60g in all but 2 trials (Littlefield et al., 2017, Chu et al., 2016). The factors that influence postprandial response such as timing, energy expenditure and intensity of exercise before OFTT will be discussed in detail later in this section.

Whilst the importance of reducing lipaemia after high fat ingestion is established, the mechanisms that mediate reductions in postprandial TG after exercise are not clear. Attenuation of postprandial lipaemia is caused by either a decrease in production of hepatic/gut TRLs or an increase in clearance of TRLs, and a combination of the two

(Freese et al., 2014). Exercise induced upregulation of lipoprotein lipase (LPL) activity or production are leading theories for greater removal of systemic TG after exercise (Freese et al., 2014, Seip et al., 1997, Kersten, 2014). However, this has been contested by an in vitro study that proposed TG removal is increased by exercise induced alterations in VLDL1 composition (Ghafouri et al., 2015). VLDL1 modification increases its affinity to LPL without upregulation of LPL (Ghafouri et al., 2015). Interestingly, gender differences in circulating VLDL concentrations between men and pre-menopausal women help to explain why VLDL modification is beneficial. Women produce fewer VLDLs, and each VLDL contains a greater TG content (Magkos et al., 2007). Consequently, these larger VLDL particles are more readily removed from the circulation. This explains the lower levels of lipaemia observed in women and may be a contributing factor to the lower incidence in CVD in women compared with men (Magkos et al., 2007). Therefore, exercise induced alterations in VLDL1 composition observed in men may be a plausible mechanism for reduced postprandial lipaemia (Ghafouri et al., 2015). In support of this mechanism, some studies have demonstrated that moderate intensity (65%  $\dot{V}O_2$ peak for 30 minutes; Miyashita and Tokuyama, 2008) and moderate intensity to high intensity (70%  $\dot{V}O_2$ peak for 90 minutes & 10x 1 minute sprints; Harrison et al., 2012) exercise does not significantly alter LPL production (Harrison et al., 2012, Miyashita and Tokuyama, 2008), however, the contrary has also been reported (Seip et al., 1997, Herd et al., 2001).

The proposed mechanisms of exercise induced reductions in postprandial lipaemia have prompted further studies to identify the optimal timing, modality, energy expenditure and intensity of exercise.

**Table 1.1** Recent studies investigating the effects of acute prior exercise and postprandial TG responses

Author (year)	N, gender	Age (years)	BMI (kg.m <sup>-2</sup> )	Mean OFFT composition	Exercise modality	Timing of exercise	EE during exercise	Exercise intervention(s) (intensity and duration)	Postprandial TG
<b>Littlefield et al. (2017)</b>	7 M	43 ±10	32 ±5	1010 kcal; 100g fat; 17g CHO; 3g protein	Treadmill running	2h before OFFT	500 kcal	Rest vs low intensity (40-50% $\dot{V}O_2R$ ; 70-90 min) vs hi (70-80% $\dot{V}O_2R$ ; 45-60 min) vs Hi EPOC with energy replacement*	Reductions in TG AUC and iAUC with all exercise interventions compared to rest (P<0.05). No differences between exercise interventions
<b>Fuller et al. (2017)</b>	12 M	23 ±2	24 ±1	1000 kcal; 98g fat; 78g CHO; 32g protein	Cycle ergometer	16 to 18h before OFFT	NR	65% $\dot{V}O_2$ peak for 45 min vs rest	16% reduction in TG AUC with exercise compared to rest (p=0.02). TG iAUC not reported
<b>Chu et al. (2016)</b>	10 M	22 ±2	26 ±3	997 kcal; 98g fat; 24g CHO; 8g protein	Cycle ergometer	14.8 ±1.2h before OFFT	NR	5 min warm-up at 30 W; interval exercise 8 s at 60% peak power, 12 s active recovery for 20 min; 5 min cool down at 30W vs rest	23% reduction in TG AUC (p=0.014), 47% reduction in TG iAUC compared to rest (p=0.04)
<b>Lopes Kruger et al. (2016)</b>	11 M	23 ±3	23 ±2	941 kcal; 52g fat; 82g CHO; 35g protein	Treadmill running	Evening before OFFT – time not specified	300 kcal	Rest vs mod intensity exercise (VT1) for 38 ±5 min vs hi intensity exercise (VT2 -10%) for 33 ±4 min	Mod & hi exercise lowered TG AUC compared to rest by 21% (p=0.02) and 29% (p<0.01), respectively. TG iAUC was 49% lower after hi exercise vs rest (p=0.01), not reported for mod exercise
<b>Kaviani et al. (2016)</b>	23; 7 F, 16 M	31 ±6	30 ±4	1452 kcal; 86g fat; 106g CHO; 66g protein per 2m <sup>2</sup> (body mass and stature not reported)	Treadmill walking	~13 h before OFFT	619 ±180 kcal	50% $\dot{V}O_2$ peak for 90 min (Ex) vs 50% $\dot{V}O_2$ peak for 90 min (Ex) + energy replaced (+10%) with LGI food vs 50% $\dot{V}O_2$ peak for 90 min (Ex) + energy replaced (+10%) with HGI food vs Rest	Ex and Ex + LGI food replacement lowered TG AUC compared to rest (p<0.05). Ex + HGI food replacement was not significantly lower than rest. TG AUC for Ex + HGI was higher than the other two exercise conditions (p ≤0.05)

Author (year)	N, gender	Age (years)	BMI (kg.m <sup>-2</sup> )	Mean OFTT composition	Exercise modality	Timing of exercise	EE during exercise	Exercise intervention(s) (intensity and duration)	Postprandial TG
<b>Emerson et al. (2016)</b>	12 M	25 ±5	28 ±2	1118 kcal; 86 g fat; 86 g CHO; Protein quantity NR	Treadmill walking	12 h before OFTT	30 min, 291 ±71 kcal; 60 min, 583 ±142 kcal	Rest vs 30 min walking at 60% $\dot{V}O_{2peak}$ vs 60 min walking at 60% $\dot{V}O_{2peak}$ . Participants given HGI snack (270 kcal) immediately after both exercise conditions	No significant differences in TG AUC or iAUC between groups.
<b>Johnson et al. (2016)</b>	12 M	23 ±3	25 ±3	1543 kcal; 79g fat; 180g CHO; 23g protein. Estimate - poorly reported in paper	Cycle ergometer	12 h before OFTT	623 ±74 kcal	60% $\dot{V}O_{2peak}$ for 60 min (note: 6 exercised above VT1 and 6 exercised below VT1) vs rest	AUC not reported. No significant differences in iAUC between conditions. Of note, increase in TG was small – mean peak 0.3 (exercise) to 0.5 (control) mmol.l <sup>-1</sup> above baseline. Only baseline 2h and 4h measured.
<b>Ghafouri et al. (2015)</b>	10 M	36 ±12	30 ±5	1277 kcal; 92g fat; 68g CHO; 25g protein	Treadmill walking	16-18 h before OFTT	793 ±69 kcal	50% $\dot{V}O_{2peak}$ for 90 min vs rest	TG AUC and iAUC not reported. Time averaged TG was lower than rest (p = 0.03).
<b>Freese et al. (2015)</b>	22 F	52 ±11	31 ±7	1021 kcal 73g fat; 59g CHO; 32g protein	Cycling exercise	14-16 h before OFTT	NR	Rest vs 4x 30 s ‘all out’ cycling sprints at a resistance of 9% fat free mass. 4 min active recovery between each sprint.	TG AUC was reduced by 16% with exercise compared to rest. (p= <0.05). No significant differences in TG iAUC between exercise and rest. Of note, testing controlled for follicular phase, only 3 h postprandial samples measured.

Author (year)	N, gender	Age (years)	BMI (kg.m <sup>-2</sup> )	Mean OFTT composition	Exercise modality	Timing of exercise	EE during exercise	Exercise intervention(s) (intensity and duration)	Postprandial TG
<b>Arjunan et al. (2015)</b>	29 M; 15 South Asian (SA) M and 14 European (E) M	SA-M, 24 ±3; E-M, 22 ±1	SA-M, 25 ±3; E-M, 23 ±2	1057 kcal; 67g fat; 85g CHO; 29g protein	Treadmill walking	~17 h before OFTT	SA-M, 372 ± 86 kcal; E-M, 410 ±82 kcal	Self-selected brisk walking speed, equated to ~50% $\dot{V}O_2$ peak for 60 min vs rest	TG AUC was 8% lower in SA-M and 10% lower in E-M (p<0.05). TG iAUC was not reported. SA-M had higher TG concentrations during the postprandial period but when adjusted for age and body mass these differences were small or did not exist.
<b>Chiu et al. (2014)</b>	9 M	24 ±0.2	NR: estimated at 24 using stature and body mass.	1156 kcal; 84g fat; 77g CHO; 23g protein	Treadmill walking	~15 h before OFTT	520 ±50 kcal	50% $\dot{V}O_2$ peak for 60 min vs 50% for 60 min with EE during exercise replaced with glucose 2 h after exercise vs rest	TG AUC was 26% and 31% lower for exercise and exercise + energy replacement than control, respectively (p<0.05). TG iAUC 39% and 32% lower for exercise and exercise + energy replacement than control, respectively (p<0.05).
<b>Trombold et al. (2014)</b>	6 M	25 ±5	NR: estimated at 24 using stature and body mass.	1214 kcal; 78g fat; 104g CHO; 24g protein	Cycle ergometer	12 h before OFTT	~897 ±144 kcal	65% $\dot{V}O_2$ peak for 60 min + 10 x 1 min at 95% $\dot{V}O_2$ peak interspersed with 10 x 1 min active recovery; vs exercise protocol + high CHO energy replacement; vs exercise protocol + low CHO energy replacement; vs rest	TG AUC for exercise and exercise + low CHO energy replacement were lower than rest (p=0.03 and 0.05, respectively). Exercise + high CHO energy replacement was lower than rest (p=0.02), but was higher than exercise + high CHO replacement (p=0.03).
<b>Kim et al. (2014)</b>	9 M	24 ±4	NR: all had body mass <30	1119 kcal; 84g fat; 77g CHO; 15g protein	Treadmill running vs treadmill walking	~14 h before OFTT	Moderate exercise, 623 ±116 kcal; walking 621 ±97 kcal	65% $\dot{V}O_2$ peak for 1 h vs 9 intermittent walking sessions between 9am and 6pm, matched for moderate exercise EE vs rest	Moderate and walking exercise modalities reduced TG AUC compared to rest (p<0.001 and p<0.04). TG iAUC was lower after moderate exercise compared to walking (P=0.02) and rest (p=0.001). TG iAUC after walking was lower than rest (p=0.048)



Author (year)	N, gender	Age (years)	BMI (kg.m <sup>-2</sup> )	Mean OFTT composition	Exercise modality	Timing of exercise	EE during exercise	Exercise intervention(s) (intensity and duration)	Postprandial TG
Canale et al. (2014)	12 M	24 ±1	25 ±1	989 kcal 65g fat; 81g CHO; 20g protein	Cycle ergometer	1 h before OFTT	NR	70% HRR for 60 min vs 5x 60 s at 100% peak WR with 225 s recovery between bouts vs 10 x 15 s at 200% peak WR with 116 s recovery between bouts	No significant differences between TG responses. Of note, measurements taken at baseline, 2h and 4h
Arjunan et al. (2013)	20 M; 10 South Asian (SA) M and 10 European (E) M	SA-M, 22 ±1; E-M, 23 ±2	SA-M, 25 ±3; E-M, 25 ±2	~1100 kcal; 70g fat; 90g CHO; 31g protein	Treadmill running	18 h before OFTT	SA-M, 792 ±149 kcal; E-M, 932 ±113 kcal	70% $\dot{V}O_2$ max for 60 min vs rest	Exercise decreased postprandial TG by 22% and 10% compared to rest in SA-M and E-M, respectively (p=0.001). There was a large interaction effect for lower TG with exercise in SA-M compared to E-M. Of note, there were two OFTT meals given 4 h apart and responses measured for 9 h

Values for age, BMI and EE during exercise are reported as mean ±SD; Abbreviations: N, number of participants; M, Male participants; F, Female participants; NR, not reported; CHO, carbohydrate; h, hours; min, minutes;  $\dot{V}O_2R$ , Oxygen uptake reserve; mod, moderate intensity exercise; hi, high intensity exercise; EPOC, excess post-exercise oxygen consumption; W, Watts; s, seconds; VT1, ventilatory anaerobic threshold; VT2, respiratory compensation point; LGI, low glycemic index food; HGI, high glycaemic index food; HRR, heart rate reserve. \*the energy replaced was calculated from the difference between the EPOC of the high intensity exercise and the EPOC of the low intensity exercise.

#### 1.4.1 Timing of exercise to reduce postprandial lipaemia

The most commonly cited mechanisms underlying the improved lipaemic responses after exercise, increased LPL and/or VLDL modification, appear to occur between 4 and 18 hours after an acute exercise session (Plaisance and Fisher, 2014, Maraki and Sidossis, 2013, Freese et al., 2014, Seip et al., 1997, Ghafouri et al., 2015). As such, there are many studies that have investigated the effects of exercise performed during this timeframe before OFTT meal ingestion (Tsetsonis et al., 1997, Ghafouri et al., 2015, Miyashita et al., 2006, Miyashita and Tokuyama, 2008, Miyashita et al., 2011). Most studies have shown exercise to be effective in reducing postprandial lipaemia, reviewed by Maraki and Sidossis (2013). Additionally, there is evidence for this time period being more effective than <4 hours before (Zhang et al., 2004) and immediately after high fat meal intake (Katsanos and Moffatt, 2004). Interestingly, exercise performed 24 hours before OFTT does not appear to reduce postprandial lipaemia, demonstrating that the acute effects of exercise are short-lived (Zhang et al., 2004).

Fewer studies have investigated the effects of exercise performed <4 hours before high fat meal ingestion or the effects of exercise performed shortly after meal ingestion. The findings from several studies and systematic review articles indicate that acute exercise after high fat meal ingestion shows the smallest effect and sometimes has no effect on reducing postprandial lipaemia (Maraki and Sidossis, 2013, Plaisance and Fisher, 2014, Martins et al., 2007). Exercise reduces gastric blood flow (Rowell et al., 1964) and as such delayed gastric emptying might be a contributing mechanism towards reductions in postprandial lipaemia in postprandial exercise studies. The findings of studies that have investigated the effects of prior exercise performed <4 hours before high fat meal ingestion are inconsistent. Exercise within 4 hours of a high fat meal either lowers (Plaisance et al., 2008, Ferreira et al., 2011, Katsanos and Moffatt, 2004, Katsanos et al., 2004, Littlefield et al., 2017) or has no effect on reducing postprandial TG after high

fat meal ingestion (Pfeiffer et al., 2005, Petridou et al., 2004, Cox-York et al., 2013, Clegg et al., 2007, Katsanos et al., 2004, Canale et al., 2014). Therefore, the role of immediate prior exercise requires further investigation in order to understand whether this modality is effective, and if it is, what mechanisms explain this effect.

#### 1.4.2 Energy expenditure

Exercise interventions requiring higher energy expenditure and more specifically, those creating a large calorie deficit appear to be important for reducing postprandial lipids, according to a recent meta-analysis of 76 studies (Freese et al., 2014). A calorie deficit can also be induced by diet restriction, however, the effects of exercise for the same calorie deficit created by diet restriction causes a much greater reductions in postprandial TG (Maraki et al., 2010). This suggests that the effects of acute exercise are more complex than simply creating a calorie deficit. Tsetsonis and Hardman (1996a, 1996b) performed two studies to evaluate the role of energy expenditure, during exercise performed approximately 16 hours before OFTT ingestion (1.3g fat and 1.2g carbohydrate per kg body mass), on postprandial lipaemia in healthy young lean men and women (Tsetsonis and Hardman, 1996b, Tsetsonis and Hardman, 1996a). The first study demonstrated that 90 minutes of exercise at a higher exercise intensity and energy expenditure was more effective in reducing postprandial lipaemia than 90 minutes of exercise at a low exercise intensity with lower energy expenditure (Tsetsonis and Hardman, 1996a). The second study included a rest (control) trial and two exercise trials where the energy expenditure was fixed at approximately 1000 kcal for both exercise trials and 90 minutes of moderate intensity exercise was compared with 180 minutes of low intensity exercise. Tsetsonis and Hardman showed that both exercise trials reduced postprandial lipaemia compared to the rest trial, but there no differences

between the two exercise trials (Tsetsonis and Hardman, 1996b). As such, Tsetsonis and Hardman concluded that energy expenditure was a key factor in reducing postprandial lipaemia. Exercise interventions that have compared a single exercise session on the day prior to OFTT with accumulated exercise bouts matched for energy expenditure have also demonstrated similar benefits, supporting the importance of energy expenditure rather than exercise intensity on reducing postprandial lipaemia (Miyashita et al., 2008, Miyashita, 2008). A recent review of the literature also discussed that these studies were important in supporting the role of energy expenditure on reducing postprandial lipaemia (Plaisance and Fisher, 2014).

Recent literature investigating the role of high intensity interval exercise (HIIE) in reducing postprandial lipaemia has been comprehensively reviewed (Burns et al., 2015). Data from these studies indicate that energy expenditure appears to be less important in reducing postprandial lipaemia (Burns et al., 2015). Some of the limitations in accurately estimating energy expenditure are highlighted later in the present thesis, however, the impact of HIIE on postprandial lipaemia may alter the perception on the importance of energy expenditure in reducing postprandial lipids. From a mechanistic viewpoint, the role of LPL in modulating postprandial lipids could explain the effectiveness of HIIE. There is some evidence in animal models which suggests that increased LPL activity may be specific to type 2 muscle fibres after muscle contraction (Hamilton et al., 1998). These fibres will be heavily utilised during HIIE and they will also be utilised to a greater extent for longer duration moderate intensity exercise compared to shorter duration moderate exercise. As such, it could be hypothesised that the continuous exercise studies with the greatest benefit, those with high energy expenditure and therefore exercise protocols at high intensities or for prolonged duration, are most effective because of type 2 muscle fibre and subsequent LPL activation and high energy expenditure is a surrogate marker of these processes. This

remains an overlooked mechanism. However, it is also known that after HIIIE there is an excess post oxygen consumption (EPOC) that may remain elevated for several hours after HIIIE (Littlefield et al., 2017). As such, energy expenditures calculated during exercise that do not account for the subsequent EPOC, may not accurately reflect the energy expended from HIIIE (Skelly et al., 2014). Further research needs to be performed to delineate the roles of exercise intensity and energy expenditure in reducing postprandial lipaemia.

#### 1.4.3 Low and moderate intensity continuous exercise

Katsanos and colleagues (2004) compared moderate intensity exercise (65%  $\dot{V}O_{2max}$ ) with low intensity exercise (25%  $\dot{V}O_{2max}$ ) matched for energy expenditure (1100 kcal). Both exercise protocols were performed 1 hour before high fat meal ingestion. Moderate intensity exercise reduced postprandial lipids by 39%, whereas low intensity exercise did not significantly alter postprandial TG metabolism (Katsanos et al., 2004). Conversely, other studies have reported no differences between moderate (63%  $\dot{V}O_{2max}$ ) and low intensity (32%  $\dot{V}O_{2max}$ ) exercise matched for a similar energy expenditure when exercise was performed on the evening before OFTT ingestion (Tsetsonis and Hardman, 1996b). Many of the moderate intensity exercise interventions are performed 12 to 18 hours prior to OFTT ingestion at approximately 60 to 65% of  $\dot{V}O_{2max}$  and lasting between 60 and 120 minutes (Gill et al., 1998, Tsetsonis et al., 1997, Tsetsonis and Hardman, 1996a, Tsetsonis and Hardman, 1996b, Dekker et al., 2010). There is some evidence to support that exercise intensities as low as 25%  $\dot{V}O_{2max}$  are effective in reducing postprandial lipids (Kim et al., 2014). Exercise at 25%  $\dot{V}O_{2max}$ , at an energy expenditure matched with 1 hour of exercise at 65%  $\dot{V}O_{2max}$ , resulted in an approximate 20% reduction in postprandial TG compared to the

control (rest) trial (Kim et al., 2014). However, exercise at a higher intensity, 65%  $\dot{V}O_{2max}$ , reduced postprandial TG to a larger extent (approximately 34% lower than the control trial) (Kim et al., 2014). In addition, thirty minutes of walking at a low intensity (approximately 42%  $\dot{V}O_{2max}$ ) led to a 16% reduction in postprandial TG after OFTT ingestion the following day. This low intensity exercise intervention was effective when exercise was performed in one 30 minute session and over ten 3-minute walking sessions during the day before the OFTT (Miyashita et al., 2008).

In summary, moderate intensity aerobic exercise appears to be the most commonly studied mode of exercise used for interventions and reduce postprandial lipaemia. This mode of exercise appears to consistently reduce postprandial lipids, particularly if it is performed 12 to 18 hours prior to an OFTT. Low intensity exercise may also be beneficial but most likely has a smaller effect on reducing postprandial lipids. Of note, few studies exist in older populations or those with impaired metabolic function.

#### 1.4.4 High intensity interval exercise (HIIE)

Interval exercise involving several bursts of high intensity exercise (lasting 6 to 240 seconds) interspersed with light exercise is also an effective strategy to reduce postprandial lipaemia but few studies have been conducted (for a recent review see; (Burns et al., 2015)). Burns and colleagues (2015) identified that 11 of 15 studies reported significant reductions in postprandial TG for both submaximal (4 of 5 studies) and supramaximal (7 of 10 studies) high intensity interval exercise modes (defined relative to  $\dot{V}O_{2max}$ ) compared to no exercise (Burns et al., 2015). When compared to moderate intensity continuous exercise, submaximal high intensity interval exercise (ranging from 115% anaerobic threshold for 3 minute intervals until 500 kcal to 85% to 95% HRmax until 900 kcal) has been shown to be similar (Ferreira et al., 2011), or

more effective (Trombold et al., 2013, Freese et al., 2014), at reducing postprandial TG. More recently, Chu and colleagues (2016) investigated the effects of 20 minutes of high intensity interval exercise (HIIE) at an intensity eliciting 60% of maximum power output for 8 seconds, interspersed with 12 seconds of active recovery (Chu et al., 2016). HIIE performed 15 hours before an OFTT resulted in a 23% and 46% reduction in 4-hour TG AUC and iAUC, respectively.

As discussed below, comparisons of energy expended during low to moderate and high intensity interval exercise are difficult. This exercise modality is preferred by some due to the shorter amount of time required to complete the session (Little et al., 2010). However, the safety of supramaximal exercise is not fully understood in sedentary populations (Eskelinen et al., 2016a). Therefore this type of exercise should be prescribed with caution. HIIE involves performing bouts of vigorous exercise interspersed with light or recovery exercise (Gillen and Gibala, 2014). Vigorous exercise is defined by an exercise intensity achieving a heart rate of 80% to 95% of maximum heart rate (Gillen and Gibala, 2014). Therefore the high intensity intervals could be performed at a submaximal intensity to lower postprandial lipaemia. This could arguably offer a safer prescription of exercise to populations at increased risk of cardiovascular events. However, there are limited studies that have investigated the influence of submaximal high intensity exercise on reducing postprandial lipaemia (Burns et al., 2015).

**Table 1.2** Lipid responses in recent exercise and postprandial lipaemia studies

Author (year)	Baseline Chol (mmol.l <sup>-1</sup> )	Baseline HDL-C (mmol.l <sup>-1</sup> )	Baseline LDL-c (mmol.l <sup>-1</sup> )	Baseline TG (mmol.l <sup>-1</sup> )	TG AUC (mmol.time[h] <sup>-1</sup> .l <sup>-1</sup> )	TG iAUC (mmol.time[h] <sup>-1</sup> .l <sup>-1</sup> )
<b>Littlefield et al. (2017)</b>	4.4 ±0.9	1.0 ±0.3	2.5 ±0.6	1.8 ±1.0	NR	NR
<b>Fuller et al. (2017)</b>	NR	NR	NR	NR	NR	NR
<b>Chu et al. (2016)</b>	4.5 ±0.7	1.0 ±0.2	3.0 ±0.6	1.6 ±0.4	Rest: 9.2 ±3.4 Ex: 7.2 ±1.9 mmol.4h <sup>-1</sup> .l <sup>-1</sup>	Rest: 2.8 ±1.9 Ex: 1.5 ±1.6 mmol.4h <sup>-1</sup> .l <sup>-1</sup>
<b>Lopes Kruger et al. (2016)</b>	NR	1.1 ±0.2	3.0 ±0.6	NR	NR	NR
<b>Kaviani et al. (2016)</b>	NR	NR	NR	NR	Rest: 18.4 ±2.6 Ex-LGI: 12.5 ±1.5 Ex-HGI: 15.9 ±1.9 Ex: 13.0 ±1.4 mmol.6h <sup>-1</sup> .l <sup>-1</sup>	Rest: 5.6 ±1.0 Ex-LGI: 4.6 ±0.7 Ex-HGI: 6.4 ±0.9 Ex: 5.7 ±0.9 mmol.6h <sup>-1</sup> .l <sup>-1</sup>
<b>Emerson et al. (2016)</b>	NR	NR	NR	1.5 ±0.7	Rest: 21.1 ±9.8 Ex-30: 20.5 ±11.7 Ex-60: 16.3 ±9.0 mmol.8h <sup>-1</sup> .l <sup>-1</sup>	Rest: 8.1 ±4.8 Ex-30: 8.3 ±5.4 Ex-60: 5.8 ±3.4 mmol.8h <sup>-1</sup> .l <sup>-1</sup>
<b>Johnson et al. (2016)</b>	3.7 ±0.6	1.1 ±0.1	2.3 ±0.4	0.6 ±0.03	NR	NR
<b>Ghafouri et al. (2015)</b>	5.4 ±0.3	1.1 ±0.1	3.7 ±0.3	1.6 (1.2 to 2.1)	NR	NR
<b>Freese et al. (2015)</b>	NR	NR	NR	NR	NR	Rest: 3.9 ±2.3 Ex: 3.2 ±2.2 mmol.3h <sup>-1</sup> .l <sup>-1</sup>



Author (year)	Baseline Chol (mmol.l <sup>-1</sup> )	Baseline HDL-C (mmol.l <sup>-1</sup> )	Baseline LDL-c (mmol.l <sup>-1</sup> )	Baseline TG (mmol.l <sup>-1</sup> )	TG AUC (mmol.time[h] <sup>-1</sup> .l <sup>-1</sup> )	TG iAUC (mmol.time[h] <sup>-1</sup> .l <sup>-1</sup> )
<b>Arjunan et al. (2015)</b>	SA-M: 4.0 (3.7 to 4.3) E-M: 3.8 (3.5 4.1)	SA-M: 0.9 (0.8 to 1.0) E-M: 1.2 (1.1 to 1.3)	NR	SA-M: 1.3 (1.0 to 1.6) E-M: 0.9 (0.7 to 1.1)	NR	NR
<b>Chiu et al. (2014)</b>	4.6 ±0.3	1.8 ±0.2	2.4 ±0.21	0.9 ±0.1	NR	NR
<b>Trombold et al. (2014)</b>	NR	NR	NR	NR	Rest: 6.6 ±2.1 Ex:3.8 ±0.4 Ex+LC: 3.7 ±0.7 Ex+HC: 5.1 ±1.3 mmol.4h <sup>-1</sup> .l <sup>-1</sup>	NR
<b>Kim et al. (2014)</b>	NR	NR	NR	1.0 ±0.1	Rest: 12.8 ±1.2 Low ex: 10.7 ±1.0 Mod ex: 9.7 ±0.8	NR
<b>Canale et al. (2014)</b>	NR	NR	NR	NR	NR	NR
<b>Arjunan et al. (2013)</b>	SA-M: 3.9 ±0.5 E-M: 3.8 ±0.8	SA-M: 1.0 ±0.2 E-M: 1.3 ±0.3	NR	SA-M: 1.4 ±0.5 E-M: 1.0 ±0.4	SA-M Rest: 26.1 ±7.1 Ex: 20.2 ±5.2 E-M Rest: 15.2 ±3.9 Ex: 13.7 ±3.7 mmol.9h <sup>-1</sup> .l <sup>-1</sup>	NR

Values are presented as mean ±SD or geometric mean (95% CI); Abbreviations: NR, not reported; SA-M, South Asian men; E-M, European men; Ex, exercise; Ex-LGI, exercise with low glycaemic index energy replacement; Ex-HGI, exercise with high glycaemic index energy replacement; Ex+LC, exercise with low carbohydrate content energy replacement; Ex+HC, exercise with high carbohydrate energy replacement; Low ex, low intensity exercise; Mod ex, moderate intensity exercise; Ex-30, 30 minutes of

## 1.5 Limitations in controlling for exercise intensity and energy expenditure

Difficulties in comparing trained and untrained groups when assessing the influence of acute exercise on postprandial lipaemia lie within the design of the exercise interventions. A frequently used “moderate” exercise intensity in exercise intervention OFTT studies is 60 to 65%  $\dot{V}O_{2\text{peak}}$  for a given period of time (Gill et al., 2002, Gill et al., 1998, Chu et al., 2016, Emerson et al., 2016, Trombold et al., 2013, Johnson et al., 2016). This has substantial limitations when comparing trained and untrained groups; primarily, as is commonly discussed, a trained individual working at the same “relative intensity” (60%  $\dot{V}O_{2\text{peak}}$ ) will perform more work and therefore expend more energy in the same time period (duration). This is an issue because it is postulated that energy expenditure is a key determinant of the effect of exercise in reducing postprandial lipaemia (Plaisance and Fisher, 2014). However, secondly, a largely overlooked area within the literature are the exercise intensity domains, particularly in this case the anaerobic threshold. It is widely accepted that a point occurs at approximately 40 to 60%  $\dot{V}O_{2\text{peak}}$  where anaerobic glycolysis becomes a significant contributor to ATP turnover (Beaver et al., 1986), which by definition can only utilise substrates glycogen and glucose during this anaerobic glycolytic process. It is also widely accepted that in endurance trained individuals, this threshold increases to a higher percentage of  $\dot{V}O_{2\text{peak}}$ , up to 80% in elite endurance athletes (Davis et al., 1979, Joyner and Coyle, 2008). Oxygen consumption during constant work exercise at an intensity above the anaerobic threshold takes longer to reach or may never reach a steady state (Poole and Jones, 2012). There is an additional oxygen cost to steady state exercise above the anaerobic threshold compared to below the anaerobic threshold, this is referred to as the ‘slow component’ (Poole and Jones, 2012). The extra oxygen cost equates to an extra energy cost, highlighting the inefficiencies of metabolic processes beyond the anaerobic threshold (Poole and Jones, 2012). In an untrained group, it is likely that the anaerobic

threshold will occur at a lower percentage of  $\dot{V}O_{2peak}$  compared to an endurance trained group (Joyner and Coyle, 2008, Davis et al., 1979). Therefore, the relative intensity of exercise and the slow component contribution to exercise between an untrained group and trained group are likely different, and even within the trained and untrained groups there are likely to be intra-individual differences.

Studies have investigated the role of energy expenditure in reducing postprandial lipaemia by defining the total energy expenditure of the exercise and varying the duration and intensity of the exercise (Tsetsonis and Hardman, 1996a). Whilst this better addresses the issue of energy expenditure, the relative intensity commonly employed remains to be a percentage of  $\dot{V}O_{2peak}$ . In these studies energy expenditure is often estimated using standard measurements of oxygen consumption and carbon dioxide production from expired gas analysis. This is an accurate method of estimating energy expenditure, by indirect calorimetry, up to the oxygen consumption at the anaerobic threshold. However, beyond the anaerobic threshold carbon dioxide (a key variable in the estimation of energy expenditure) is produced from non-substrate related processes (buffering of hydrogen ions with bicarbonate and hyperventilation during metabolic acidosis). The calculations used to estimate energy expenditure with expired gas analysis assume that the expired  $CO_2$  measured is a result of aerobic substrate metabolism (Weir, 1949). As such the accuracy of the calculation must decrease considerably when exercise is performed beyond the anaerobic threshold. This issue is highlighted in the postprandial study by Johnson and colleagues (2016); participants were exercised at 60%  $\dot{V}O_{2peak}$  and the authors reported that this led to 6 participants exercised at an intensity above the anaerobic threshold and 6 participants exercised at an intensity below the anaerobic threshold. The authors raised this issue and reported a mean estimated energy expenditure for their participants during the exercise intervention, however, the authors did not explain if or how their estimation of energy

expenditure was altered for those exercising above the anaerobic threshold. When designing exercise interventions, exercise intensity could be prescribed relative to the energy intensity domains to improve likelihood that the intensity of the exercise intervention is “relative” across participants. For example, a percentage of the difference between anaerobic threshold and  $\dot{V}O_{2peak}$  could be employed, as has been performed in oxygen uptake kinetics studies (Poole and Jones, 2012). However, the author is not aware of studies that have designed exercise interventions using this method within this field of research.

## 1.6 Postprandial lipaemia in conditions of obesity and those with a family history of type 2 diabetes

### 1.6.1 Obesity and cardio-metabolic disease

Obesity is at epidemic levels, it was estimated in 2014 that 13% of adults worldwide were obese, continuing the upward trajectory in the prevalence of obesity (World Health Organisation, 2017). Obesity is strongly related to dyslipidaemia, insulin resistance, T2D, CVD and reduced life expectancy (Bell et al., 2014, Jung and Choi, 2014, Poirier et al., 2006). Dietary habits and physical activity are key aspects of lifestyle modification in obesity. In its simplest form, increases in body mass are caused by a higher energy intake compared to energy expenditure (Swinburn et al., 2009). Energy intake is determined by food consumption over a period of time, energy expenditure is the sum of energy utilised for daily living in addition to the energy expended in physical activity. Therefore, with modification of energy intake, through diet, and/or energy expenditure, with exercise, one can aim to achieve an equilibrium in order to maintain weight.

A prolonged imbalance, where calorie intake supersedes expenditure will lead to gains in body mass and may lead to obesity. High intake of fat as part of this imbalance leads to increases in the size of adipose tissue cells, leading to insulin resistance (Frayn, 2002). There is a strong relationship between increased fat mass and hypertriglyceridaemia, even in those who are not obese (defined by a BMI  $\leq 30$  kg.m<sup>-2</sup>) (Frayn, 2002). In obese individuals, the capacity to suppress systemic release of fatty acids becomes impaired, in addition, dysregulation of LPL impairs capacity to clear systemic TGs (Frayn, 2002, Panarotto et al., 2002). There are also increases in de novo lipogenesis within enterocytes (Steenenson et al., 2017) which contribute to overproduction of Apo B48 and CM secretion in centrally obese men (Wong et al., 2014). Hepatic VLDL production is also increased in the postprandial period in insulin resistant men (Shojaee-Moradie et al., 2013). It is not surprising therefore that dyslipidaemia (characterised by high TG, and small dense LDL particles, and low HDL-c) is the most common feature in obesity and insulin resistance (Wong et al., 2014). Or that people with increased fat mass have more pronounced lipaemic responses to high fat meals (Frayn, 2002). Due to the increased risk of postprandial lipaemia within obesity, this group is of interest when identifying interventions to reduce postprandial dysfunction. It would also be sensible to target other populations at risk of insulin resistance such as overweight and inactive cohorts and those who have a family history of type 2 diabetes.

### 1.6.2 Family history of T2D: a risk factor for cardio-metabolic disease

The risks of developing T2D are increased considerably in people where one or both biological parents have T2D (OT2D) (Hariri et al., 2006). Genetic, behavioural and environmental factors appear to contribute to this risk (Scott et al., 2013). The argument

for behavioural and environmental factors being key to developing T2D in OT2D has been supported by the 26% increased risk observed in partners (spouses) of an individuals with T2D (Leong et al., 2014, Khan et al., 2003). Conversely, OT2D adopted during childhood by parents without T2D have the same increased T2D risk as OT2D living with their biological parents (Hemminki et al., 2010). This observation supports the genetic contribution to T2D development in OT2D (Hemminki et al., 2010). Attributing causal factors for the progression of T2D within this population is therefore challenging. A recent study identified that established risk factors relating to lifestyle, anthropometric measures and genetic risk factors (assessing genes that have polymorphisms associated with T2D risk) only explained a small amount of inherited risk (Scott et al., 2013). Nonetheless, family history remained a strong independent predictor of T2D (Scott et al., 2013). It is therefore important that more is understood with regard to identifying early pathophysiological changes associated with increased cardio-metabolic risk in OT2D.

The apparent increased risk of developing T2D within offspring has prompted several studies to investigate whether metabolic irregularities occur in OT2D. In OT2D, central adiposity, dyslipidaemia, reduced physical fitness and other early indications of metabolic impairment are present prior to development of T2D (Nyholm et al., 2004, Cnop et al., 2007, Madec et al., 2011). These metabolic perturbations are of interest because they are associated with increased risk of CVD and T2D. Therefore, early preventative strategies are potentially important in this population in order to reduce disease progression, healthcare burden and improve quality of life. OT2D may also serve as a useful cohort to further understand mechanisms of T2D progression as they exhibit early metabolic dysregulation but do not have the various confounding co-morbidities associated with T2D.

Axelsen and colleagues (1999) compared normoglycaemic 1<sup>st</sup> degree relatives of people with T2D (n=13) to matched controls (n=13) after a mixed meal ingestion (51g fat and 83g carbohydrate) (Axelsen et al., 1999). Relatives had higher glucose at baseline and at the 1 hour postprandial time-point, postprandial insulin was higher at 3-hour and 4-hour time-points. In contrast, AUC for 6 hour glucose and insulin were not significantly different between the OT2D and control groups after the OFTT. Despite similar fasting TGs, postprandial TGs were 50% higher in relatives compared to controls. Pietraszek and colleagues (2014) also identified a greater postprandial TG response after OFTT in 1<sup>st</sup> degree relatives compared with a control group after adjusting for age, gender, body fat percentage and BMI (Pietraszek et al., 2014). Johanson and colleagues (2004) investigated the postprandial secretion of lipoproteins derived from the intestine (Apo B-48) and the liver (Apo B-100) after a high fat meal (51g fat, 83g carbohydrate) in OT2D and in healthy controls (Johanson et al., 2004). They observed increases in both Apo B-48 (50%) and Apo B-100 (21%) VLDL1 secretion after a high fat meal in OT2D compared to a matched control population. Apo B-100 secretion rates were inversely related to the degree of insulin resistance in OT2D. This highlights the relationship between insulin resistance and postprandial lipaemia in addition to the increased risk of metabolic disease progression in OT2D. Furthermore, Apo B-100 and Apo B-48 containing lipoproteins are related to atherosclerosis development (Nakajima et al., 2011, Karpe et al., 1994, Proctor et al., 2000), such evidence therefore supports the increased CVD risk in this OT2D cohort. It is important to note that fasting blood lipids as well as lifestyle factors were not different between groups. Accordingly, the postprandial response to a high fat meal detects early changes in TG metabolism which would go unnoticed with current standard clinical assessments.

In healthy individuals, several studies have indicated that the lipaemic response following a high fat meal (80g fat) is reduced if the carbohydrate content in the meal is

high (100g carbohydrate) compared to low (20g carbohydrate) (Kriketos et al., 2003, Kriketos et al., 2005, Westphal et al., 2002). Kriketos and colleagues (2005) investigated the effects of two high fat meals with differing carbohydrate content in OT2D (normoglycaemic and normolipidaemic) and healthy controls matched for BMI and age (Kriketos et al., 2005). OT2D were more insulin resistant, determined by a hyperinsulaemic-euglycaemic clamp. Normoglycaemic and normolipidaemic groups had similar postprandial triglyceride responses to the control group after the high fat meal with low carbohydrate content. The two groups differed in response to the high fat meal with a high carbohydrate content; the control group had a markedly lower postprandial TG response when compared to the high fat – low carbohydrate meal. Furthermore, the control group showed lower postprandial TG responses compared to the responses of OT2D to both meals. Interestingly, OT2D had similar TG responses to both high fat meals irrespective of carbohydrate content. In addition, nonesterified fatty acids (NEFA) were suppressed to a larger extent in the high fat – high carbohydrate meal compared to the high fat – low carbohydrate meal. There were higher insulin responses to the high fat – high carbohydrate meals in both groups. Insulin has been shown to be a key regulator of VLDL production (Pirillo et al., 2014). Therefore, these findings indicate that in OT2D, who were more insulin resistant, increases in TG concentrations was not suppressed by the increased insulin concentrations with high carbohydrate, high fat intake. Given the strong association between acute exercise and increased insulin sensitivity, it would be interesting to investigate whether improving insulin sensitivity would improve the postprandial TG response to high fat high carbohydrate meals in OT2D. Such a study would provide further insight to the mechanisms of acute exercise reducing postprandial lipaemia. However, to the author's knowledge this has not been investigated.



Although differences in postprandial TGs have been observed between 1<sup>st</sup> degree relatives and healthy controls after OFTT ingestion, the evidence for differences in postprandial markers of inflammation is less clear. Pietraszek and colleagues (2011) reported that there were no differences in postprandial inflammatory markers after high fat meal (82g fat, 23g carbohydrate, 4g protein) ingestion between 1<sup>st</sup> degree relatives and matched controls (Pietraszek et al., 2011). IL-6 was increased in both groups, however, there were no differences between the two groups (Pietraszek et al., 2011). Madec and colleagues (2011) investigated the effects of high fat meal (43g fat, 64g carbohydrate, 24g protein) consumption on inflammatory markers in 1<sup>st</sup> degree relatives of people with type 2 diabetes (Madec et al., 2011). Contrary to the studies discussed above, the TG and the insulin responses increased similarly in both groups after meal consumption. There were also no differences in real time PCR expression of IL-6, IL-8 or IL-1 $\beta$  or plasma IL-6 before and after the high fat meal in the 1<sup>st</sup> degree relatives or the control group. However, markers of endothelial function; vascular adhesion molecules; VCAM-1 and ICAM-1, and nitrotyrosine were elevated after the meal in the 1<sup>st</sup> degree relatives but not the control group. As such, inflammatory responses to high fat meals do not appear to differ between 1<sup>st</sup> degree relatives of T2D and apparently healthy controls. However, endothelial dysfunction, a precursor to CVD (Siti et al., 2015), may be more pronounced in 1<sup>st</sup> degree relatives. Reducing postprandial lipid excursions may therefore be beneficial in OT2D to improve cardio-metabolic health.

### 1.7 Polyphenol supplementation and the Strawberry (*Fragaria x ananassa*)

This review of the literature has highlighted the significance of exposure to elevated postprandial lipids, introduced groups at increased risk of cardio-metabolic disease, and the role that exercise may play in reducing postprandial lipaemia. Similar to exercise,

nutritional interventions using polyphenol rich foods have been shown to positively modulate postprandial responses after fat ingestion (Burton-Freeman et al., 2010, Edirisinghe et al., 2011). This section will introduce the role of polyphenol intake in attenuating cardio-metabolic risk with a focus on the seminal studies in the area of polyphenol ingestion and postprandial lipid metabolism.

A phenol is a compound with a phenyl ring, containing 6 Carbon atoms with alternating double and single bonds (aromatic carbon) and 5 Hydrogen atoms, which is bound to a hydroxyl (Oxygen-Hydrogen) group. Polyphenols are chemical compounds containing more than one phenol group and >8000 different compounds exist (Pandey and Rizvi, 2009). Polyphenols are particularly abundant in foods such as cocoa, fruits and vegetables and drinks such as tea and red wine. Interest in polyphenols, from a public health perspective, has increased over the past 20 years due to the association between increased intake of these foods and reduced incidence of cardiovascular events (Hertog et al., 1995, Khan et al., 2014, Tangney and Rasmussen, 2013, Sofi et al., 2014). Several theoretical mechanisms exist as to how polyphenols reduce cardiovascular risk such as; reducing inflammation, inhibition of platelet aggregation, reducing postprandial lipaemia, reducing LDL particle oxidation and increased scavenging of free radicals (Basu et al., 2014b).

In vitro, certain groups of polyphenols such as flavonoids have been shown to have a great capacity to scavenge reactive oxygen species, highlighting their antioxidant capacity (Halliwell, 2008). These experiments are often criticised because the volumes of polyphenols used are considerably greater than the bioavailability of polyphenols in humans after consumption of foods high in polyphenols. It is therefore argued that due to the limited bioavailability of polyphenols it would appear unlikely that their antioxidant capacity would sufficiently reduce oxidants within the human body

(Halliwell, 2008). However, oxidation of LDL is a key mechanism of atherosclerosis and consumption of foods high in certain antioxidants lower circulating oxLDL after fatty meal consumption (Burton-Freeman et al., 2010, Aviram et al., 2000). After consuming 100g of fruits such as blueberries, blackberries and strawberries, the total antioxidant capacity of blood plasma is increased by 1000 to 3000  $\mu\text{mol}$  (Harasym and Oledzki, 2014).

Polyphenolic compounds, particularly anthocyanins, within berries are of particular interest due to the inverse epidemiological relationships between berry consumption and CVD (Cassidy et al., 2013) and developing type 2 diabetes (Wedick et al., 2012). Of the berries, the strawberry has been identified as having the greatest antioxidant capacity (Wang and Lin, 2000, Proteggente et al., 2002), this may be an important property of the strawberry associated with reducing cardio-metabolic disease risk (Basu et al., 2014b). From a nutritional perspective, the strawberry provides; fibre, fructose, essential fatty acids, carotenoids, vitamin C, folate, manganese (Giampieri et al., 2012). Arguably more importantly from a disease prevention perspective, the strawberry may contain around 40 types of non-nutritional polyphenols (Giampieri et al., 2012). These include anthocyanins, elagitannins and quercetin, which are thought to be important for disease prevention. The amount of polyphenols available in each berry are affected by how ripeness at harvest, environmental conditions/exposures during ripening, processing methods and storage (Pandey and Rizvi, 2009).

Short-term supplementation of 50g freeze dried strawberries reduced cardiovascular risk factors, including blood pressure and total cholesterol, in people with T2D (Amani et al., 2014). In healthy individuals, 30 days of strawberry consumption (500g fresh strawberries per day) improved lipid profile, antioxidant capacity and platelet function (Alvarez-Suarez et al., 2014). Basu and colleagues showed reduced LDL-c and lipid

peroxidation after 12 weeks of freeze dried strawberry supplementation (Basu et al., 2014a). Acute studies of strawberry supplementation have also shown promising results, Tulipani and colleagues (2009) investigated the effects of consuming 1kg of one of 6 different varieties of fresh strawberries on plasma oxidative capacity in eight participants (Tulipani et al., 2009). After an overnight fast and refraining from foods and beverages high in polyphenols for 12 hours participants had a blood sample taken, consumed the strawberries within 10 minutes and then had blood samples taken each hour for 3 hours after consumption (Tulipani et al., 2009). Total antioxidant capacity of plasma increased with consumption of strawberries regardless of strawberry variety, however, there were differences in total antioxidant capacity between the different strawberry varieties, with the lowest observed in the nutritionally poorest cultivar. A decrease in serum TGs from baseline by  $0.2 \text{ mmol.l}^{-1}$  was also observed 2 hours after strawberry intake. Cao and colleagues (1998) also showed a 10 to 13% increase in antioxidant capacity in serum 4-hour AUC after 240g fresh strawberry consumption in eight healthy elderly female participants (Cao et al., 1998). These studies demonstrate the capacity for different varieties of strawberries to increase circulating antioxidant capacity.

Burton-Freeman and colleagues (2010) investigated the effects of acute and short-term supplementation with a strawberry beverage containing 10g of freeze dried strawberries in twenty four overweight hyperlipidaemic men (n= 10) and women (n= 14) (Burton-Freeman et al., 2010). After 7 days without consuming berries, participants consumed a standardised meal and either the strawberry beverage or the placebo drink and then returned 3 days later to consume the drink which they did not consume on the initial day. The test meal was a mixed meal containing 962 calories and 30.7g fat (14.6g saturated), 135.5g carbohydrate, 36.5g protein. TGs, HDL-c, and oxLDL particles were significantly lower in the postprandial state with the strawberry beverage compared to

the placebo ( $P=0.005$ ,  $P=0.003$ ,  $P=0.0008$ , respectively). Interestingly, LDL-c was affected by the strawberry beverage only in men, as was postprandial oxLDL.

Since this initial study, this research group have established that acute strawberry intake can lower inflammation (CRP and IL-6) and improve insulin sensitivity after moderate fat, high carbohydrate ingestion (Edirisinghe et al., 2011). Additionally, they have identified that the optimal dose for lowering postprandial oxidised LDL was 20g when they compared postprandial responses to a high fat – high carbohydrate meal with 0g 20g and 40g of freeze dried strawberries (Park et al., 2016). The antioxidant and anti-inflammatory properties of strawberries that reduce postprandial cardio-metabolic dysfunction are interesting and the mechanisms involved appear to be different from the exercise induced postprandial benefits after high fat meal ingestion. Acute exercise and acute strawberry ingestion in combination could therefore enhance the amelioration of cardio-metabolic dysfunction that ensues following fat ingestion, over and above the effects that either intervention may elicit alone. However, to the authors' knowledge this has not been investigated.

## 1.8 Summary and thesis aims

In obesity and insulin resistant states, after fat ingestion, the postprandial period encompasses systemic lipaemia, inflammation, oxidative stress and subsequent increased cardio-metabolic risk (Pirillo et al., 2014). Greater postprandial lipaemic excursions are predictive of CVD (Nordestgaard et al., 2007, Sarwar et al., 2007) and are a common feature of type 2 diabetes (Ginsberg and Illingworth, 2001). This can be assessed by an OFTT. However, the test-retest error of a test must be known prior its use for serial follow up, or to assess efficacy of medical or lifestyle interventions. Few studies that have evaluated the test-retest repeatability of the OFTT, and none have

assessed the repeatability of the OFTT recommended by the expert panel. A study to evaluate the repeatability of the OFTT in keeping with recommendations from an expert panel (Kolovou et al., 2011) is therefore required prior to its use in intervention studies.

Exercise performed 12-18 hours before an OFTT has been consistently shown to reduce postprandial lipaemia and subsequent inflammation, oxidative stresses and endothelial dysfunction (Peddie et al., 2012, Teeman et al., 2016). However, the effects of exercise performed within 4 hours of OFTT appear inconsistent (Plaisance et al., 2008, Ferreira et al., 2011, Katsanos and Moffatt, 2004, Katsanos et al., 2004, Pfeiffer et al., 2005, Petridou et al., 2004, Cox-York et al., 2013). To better understand this, the consistency of the effect of exercise <4 hours before could be assessed by repeat testing using the same dose of exercise and the same OFTT meal within the same person. However, no study to the author's knowledge has investigated the consistency of the effect of exercise on OFTTs.

Several plausible mechanisms exist for the exercise induced reductions in postprandial lipaemia. These include; increased LPL activity (Seip et al., 1997, Kersten, 2014), reduced hepatic production of VLDL and modification of VLDL; increasing its affinity to LPL (Ghafouri et al., 2015). One mechanism that appears to have been dismissed, is the role of exercise induced improvements in insulin sensitivity on postprandial fat metabolism (Gill et al., 2002). However, this has only been assessed in pooled data for apparently healthy people who consumed high fat – high carbohydrate OFTT (Gill et al., 2002). A high carbohydrate content (excluding fructose) OFTT provokes a lower postprandial lipaemic response compared to an OFTT with the same amount of fat and a lower carbohydrate content OFTT (Kriketos et al., 2005). This is probably due to increases in circulating insulin concentrations reducing either hepatic VLDL production or increasing LPL activity. Importantly, this effect is not observed in apparently healthy

OT2D, which may be caused by some degree of underlying insulin resistance (Kriketos et al., 2005). Insulin is a key regulator of hepatic VLDL production and LPL, and importantly, LPL activity is suppressed in people with insulin resistance (Panarotto et al., 2002). Furthermore, exercise acutely improves insulin sensitivity (Rabøl et al., 2011). It would therefore be interesting to identify whether exercise can acutely improve postprandial responses to OFTT containing high carbohydrate (and subsequent high postprandial insulin) and compare these responses to OFTT with low carbohydrate content (and subsequent low postprandial insulin) in OT2D. Firstly, this would give insight in to reducing postprandial cardio-metabolic dysfunction in OT2D. Secondly, it may give insight in to the role of insulin and insulin resistance in exercise induced reductions in postprandial lipaemia; an area that has been largely dismissed due to a retrospective analysis on pooled study data from one institution (Gill et al., 2002).

Finally, postprandial responses to cardio-metabolic dysfunction after OFTT are reduced with strawberry supplementation (Burton-Freeman et al., 2010, Edirisinghe et al., 2011). The mechanisms involved in reducing postprandial dysfunction in postprandial lipid metabolism appear different to those involved with exercise induced reductions in postprandial dysfunction. It is plausible therefore that acute exercise and acute strawberry ingestion combined could elicit greater benefits in reducing postprandial dysfunction. To the author's knowledge this has not been investigated previously.

In summary, the series of studies presented in this thesis aim to identify a repeatable OFTT meal using recommendations from an expert panel. Evaluate the consistency of the effects of exercise in reducing postprandial lipid excursions. Explore mechanisms related to exercise induced improvements in insulin sensitivity and postprandial fat metabolism with high and low carbohydrate OFTT in a group at increased risk of cardio-metabolic disorders, namely apparently healthy OT2D. Finally this body of work

will investigate the effects of separate and combined exercise and a strawberry nutritional intervention to acutely reduce postprandial cardio-metabolic dysfunction in overweight/obese men.

Study aims:

1. Assess the test-retest repeatability of an OFTT containing the macronutrients recommended by an expert panel (Kolovou et al., 2011). (Chapter 3)
2. Identify whether the effects of a fixed dose of acute moderate intensity exercise (90% anaerobic threshold) performed immediately before OFTT are consistent and effective in reducing postprandial lipaemia. (Chapter 3)
3. Evaluate the effect of acute moderate intensity exercise (90% anaerobic threshold) and OFTT carbohydrate content on postprandial lipaemia responses in OT2D. (Chapter 4)
4. Evaluate the individual and combined effects of acute exercise and strawberry ingestion on postprandial lipaemia and oxidative stress after OFTT. (Chapter 5)



## Chapter 2: General Methods

This chapter will discuss the general methods that are common to the three experimental studies included in this thesis. Aims, hypotheses and statistical analyses for each individual study are not included in this Chapter.

### 2.1 Participants & recruitment

Apparently healthy adult males were recruited for these studies. Study 1 included apparently healthy males. Study 2 included apparently healthy adult males whose biological parents (one or both) had been diagnosed with type 2 diabetes (OT2D). Study 3 included apparently healthy males who were inactive (defined by <150 minutes of exercise per week) overweight/obese (defined by a BMI >25 kg.m<sup>-2</sup> and waist circumference >94 cm). Participants were excluded if they had a past medical history of CVD, gastrointestinal disease, liver disease, lipid lowering medication, hypertension, smoking or diabetes. Studies were advertised within the University of Hull by means of posters on notice boards, emails and in a weekly staff newsletter. Studies were also advertised on social media, and for study 2 the study information was presented at a local patient group meeting for people living with type 2 diabetes. All studies were approved by the Department of Sport, Health and Exercise Science Ethics Committee, University of Hull (See Appendices 1, 3 and 4, for ethical approval documents for chapters 3, 4 and 5, respectively). All participants provided their written informed consent prior to study commencement and at least 24 hours after they had received the participation information sheet.

## 2.2 Study design

All studies included one screening visit and 4 experimental conditions investigating postprandial lipaemic responses (serum TG) after OFTT (detailed below). For all studies, participants attended the research laboratory before 10:00am for the 4 OFTT experimental conditions. Each study visit was separated by at least 72 hours. The order in which the trial conditions were performed was randomised for each participant (Research Randomizer Version 4.0, Urbaniak & Plous 2013). Participants refrained from alcohol, exercise and caffeine 24 hours before each visit and attended the research laboratory having fasted overnight. Meal intake was standardised on the evening prior to each experimental condition by providing participants with a standardised “ready meal”, detailed below. All tests were completed within 8 weeks of the screening visit.

## 2.3 Screening visit

### 2.3.1 Stature

Stature was measured to the nearest mm with the participants standing upright in the standard anatomical position (Frankfort plane) against a wall-mounted stadiometer (Harpenden Stadiometer, Holtain Limited, Crymych Pembrokeshire). Participants were encouraged to perform a deep inhalation and the measurement was recorded.

### 2.3.2 Body mass

Throughout study 1, body mass was measured on Seca 635 platform scales (Hamburg, Germany) to the nearest 0.2 kg. Throughout studies 2 and 3, body mass was measured on Seca Balance Scales (Hamburg, Germany) to the nearest 0.1 kg.

### 2.3.3 Body composition measures

Waist & hip circumferences (Seca 201 ergonomic circumference measuring tape, Hamburg, Germany) were measured in all studies. Measurements were taken in duplicate and the mean was reported, further measures were taken if there was a discrepancy by more than 1 cm between measures. Waist circumference measurements involved a horizontal measurement taken directly above the iliac crest (Pescatello and American College of Sports Medicine, 2014). Hip circumference measurements were taken at the largest portion of the buttocks on a horizontal plane (Pescatello and American College of Sports Medicine, 2014). Body fat percentage (BF900 Maltron Body Composition Analyser, Essex, UK) was measured and recorded using bioimpedence analysis and the manufacturers standard equations that adjust body fat estimates for age, ethnicity, body mass, stature and activity status. Participants were supine with their legs slightly apart and their right wrist approximately 3 inches away from the body. Electrodes were placed in the standard tetrapolar arrangement. On the right side of the participant; one electrode was placed on the wrist and the other on the hand just before the metacarpo-phalangeal joint in the second space between the metacarpals. One electrode was placed on the ankle joint between the lateral and medial malleoli and the other placed on the foot just before the metatarso-phalangeal joint in the second metatarsal space.

### 2.3.4 Oral glucose tolerance test (OGTT)

The screening visit for study 1 (chapter 3) and study 2 (chapter 4) included a 2 hour oral glucose tolerance test (OGTT). Participants attended the laboratory before 10am after an overnight fast. A cannula was inserted in to a vein in the lower arm of the participant and a baseline blood sample was drawn. Seventy five grams of dextrose diluted in

300ml of water was orally ingested by the participant within 5 minutes. Blood samples were drawn at 30 minute intervals for 120 minutes after ingestion. Blood was separated using a centrifuge (see section 2.4.6), aliquoted and stored in a -80°C freezer until glucose (plasma) and insulin (serum) analyses were performed on these samples. Whole body insulin sensitivity was estimated from relevant insulin and glucose measurements during the oral glucose tolerance test using the Matsuda index (Matsuda and DeFronzo, 1999). This method is strongly correlated ( $r= 0.73$ ) with the hyperinsulinaemic-euglycaemic clamp.

### 2.3.5 Cardiopulmonary exercise test (CPET)

Immediately before the CPET measurement, the online breath by breath expired gas analyser was calibrated for; ambient pressure using a Fortin Barometer (Darton, London, UK), gas concentration using a 2 point gas calibration, and flow volume using a 3 litre syringe (Hans Rudolph 5530, USA). The ambient pressure detected by the gas analyser was checked against barometer pressure (and recalibrated if  $>1$  mbar error). The 2 point gas calibration was performed by calibrating the gas analyser to ambient air (20.93% Oxygen, 0.03% Carbon Dioxide) and calibration gas (17.23% Oxygen and 5.11% Carbon Dioxide), calibration checks were performed and the calibration was considered successful if ambient Oxygen, and calibration gas Oxygen and Carbon Dioxide measurement error was  $\leq 0.05\%$  and ambient Carbon Dioxide measurement error was  $\leq 0.02\%$ . Flow volume was calibrated using a 3 l syringe at a rate of  $1 \text{ l}\cdot\text{s}^{-1}$  and checked against flow rates of  $0.5 \text{ l}\cdot\text{s}^{-1}$ ,  $1 \text{ l}\cdot\text{s}^{-1}$  and  $3 \text{ l}\cdot\text{s}^{-1}$ , calibration was considered successful if inspired and expired air at all flow rates had a measurement error of  $<0.1 \text{ l}$ .

Participants performed an incremental ramp-based CPET to volitional exhaustion on an electronically braked cycle ergometer (eBike ergometer, GE Healthcare, Freiburg,

Germany) according to standard guidelines (Balady et al., 2010). Online breath by breath expired gas analysis (Cortex Metalyzer 3B, Leipzig Germany), and 12 lead ECG (GE CASE system, GE Healthcare, Freiburg, Germany) was recorded throughout the test. Automated plethysmographic Blood pressure measurements (SunTech Tango, SunTech Medical, North Carolina, USA) were recorded every 2 minutes throughout exercise and recovery. The CPET protocol involved 3 minutes of rest while seated on the cycle ergometer, 3 minutes of cycling exercise against no added resistance and 8 to 12 minutes of individualised incremental ramped exercise to exhaustion. The ramp increment was preselected by an experienced technician such that each participant would achieve 8 to 12 minutes of ramped exercise. Ramp increments ranged between participants from 15 and 40 Watts per minute ( $\text{W}\cdot\text{min}^{-1}$ ). A cadence between 60 and 90 revolutions per minute (RPM) was self-selected by each participant and maintained throughout the test.

Peak oxygen consumption ( $\dot{V}\text{O}_{2\text{peak}}$ ) was determined by identifying the highest period of oxygen consumption achieved by each participant averaged over 30 seconds (Midgley et al., 2007). The ventilatory anaerobic threshold (AT) was determined using the modified V-slope method (Beaver et al., 1986) and confirmed with the ventilatory equivalents method (Whipp et al., 1986).

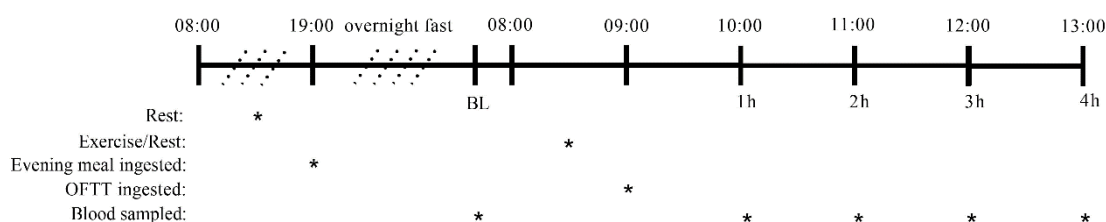
## 2.4 Experimental study visits

### 2.4.1 Visits 1-4

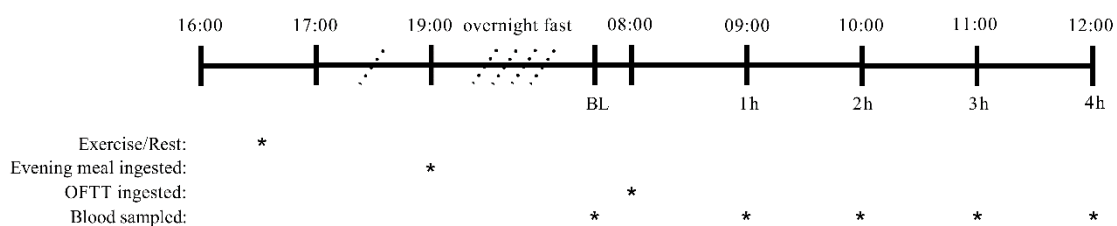
Participants were asked to consume similar breakfast and lunchtime meals at a similar time on the day prior to each visit. During studies 2 (chapter 4) and 3 (chapter 5), participants performed approximately 1 hour of exercise (during the exercise conditions

only) on the afternoon/evening before the OFTT. An evening meal was provided by the research team and consumed by the participant at home (and unsupervised) on the evening before each laboratory visit. Participants fasted overnight (>10 hours) and attended the laboratory the following morning.

The baseline measures taken on the screening visit (detailed above) were repeated. During study 1 (chapter 3), participants performed steady state exercise, during the exercise conditions only, and consumed a high fat meal immediately afterwards. Blood samples were taken 1, 2, 3 and 4 hours after the high fat meal was consumed. Schematic diagrams of study 1 (chapter 3) and studies 2 and 3 (chapters 4 and 5) are provided below in *Figure 2.1* and *Figure 2.2*, respectively.



**Figure 2.1** Study 1 (chapter 3) experimental protocol



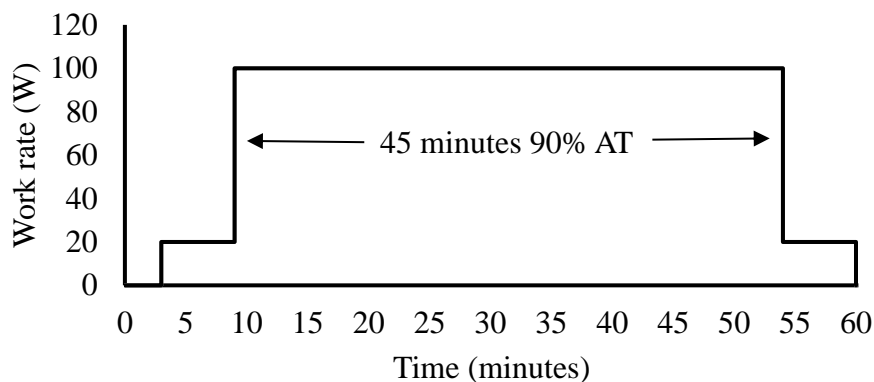
**Figure 2.2** Study 2 (chapter 4) and Study 3 (chapter 5) experimental protocol

## 2.4.2 Steady State Exercise

In study 1 and 2, before two of the four study visits, 57 minutes of aerobic exercise was performed on a cycle ergometer (eBike ergometer, GE Healthcare) using individualised

protocols (*Figure 2.3*). Exercise was performed immediately before OFTT in study 1. Exercise was performed 16-18 hours before study 2. Online breath by breath expired gas analysis (Metalyzer 3B, Cortex, Leipzig, Germany) and heart rate (Polar heart rate monitor) was measured throughout rest and exercise. The protocol required participants to cycle at 20 Watts (W) for 6 minutes, then work rate was quickly increased to elicit 90% of oxygen consumption at anaerobic threshold (90%AT) and this was maintained for 45 minutes, a 6 minute “cool down” at 20W was then performed.

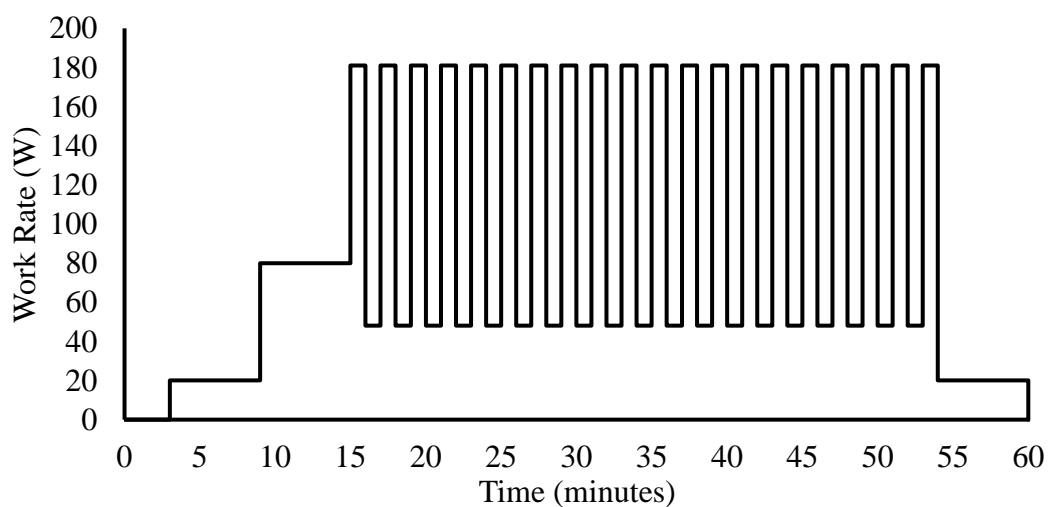
The work rate eliciting 90%AT was calculated by identifying the work rate at AT from CPET as described above, subtracting two thirds of the ramp rate from the work rate at AT to account for discrepancy between external work, muscle energetics and expired oxygen measured at the mouth (Whipp et al., 1981), and calculating 90% of this value. The same cadence that was self-selected during CPET was encouraged during steady state exercise. Ninety percent of  $\dot{V}O_2$  at the anaerobic threshold was chosen because it is nearing the upper limit of the moderate intensity exercise domain (Poole and Jones, 2012). Expired gasses were sampled throughout the exercise intervention, Oxygen consumption and Carbon Dioxide measurements were used to estimate energy expenditure using the equations proposed by Jeukendrup and Wallis (Jeukendrup and Wallis, 2005).



**Figure 2.3** Steady state exercise protocol

### 2.4.3 High intensity interval exercise (HIIE)

Submaximal high-intensity interval exercise (HIIE) was performed on a cycle ergometer (eBike ergometer, GE Healthcare) using the same individualised protocols for each of the two exercise sessions. Before interval exercise, a warm-up was performed which involved 6 minutes of continuous exercise at 20W immediately followed by 6 minutes of continuous exercise at a work rate selected at 90% of the oxygen consumption at the AT. The low-intensity interval exercise was set at 50% of the work rate at the AT. The high-intensity interval exercise was set at 50% of the difference between work rates at AT and  $\dot{V}O_{2peak}$ . The interval exercise protocol involved 1 minute of high-intensity exercise followed by 1 minute of low intensity exercise, this was repeated for 40 minutes (*Figure 2.4*). Work rates were selected by identifying the work rate at AT and  $\dot{V}O_{2peak}$  from CPET and subtracting two thirds of the ramp rate, using the same methods as the steady state exercise test. Heart rate and work rate was measured throughout the exercise protocol. Expired gasses were not measured because of the difficulties with estimating energy expenditure during exercise above the anaerobic threshold, discussed in more detail in the introduction (chapter 1.5) and in the discussion section of Chapter 5.



**Figure 2.4** HIIE exercise protocol



#### 2.4.4 Evening meal

The nutritional composition of the meal consumed on the evening before OFTT influences the postprandial response to OFTT (Robertson et al., 2002). To control for this, participants were provided with a standardised commercial meal (Tesco, UK) to consume on the evening before each OFTT study visit. In study 1 and study 2, participants chose one of three evening meals (Chicken Tikka Masala, Macaroni Cheese or beef lasagne), in study 3, participants chose 1 of 2 evening meals (Chicken Tikka Masala or Macaroni Cheese). The meals had similar nutritional and calorie composition. The same meal was consumed by the participant on the evening before all OFTT experimental trials.

**Table 2.1** Mean  $\pm$ SD evening meal composition for each study

	<b>Study 1</b>	<b>Study 2</b>	<b>Study 3</b>
<b>Energy (kcal)</b>	738 $\pm$ 18	756 $\pm$ 13	756 $\pm$ 13
<b>Fat (g)</b>	33 $\pm$ 2.0	33 $\pm$ 0	33 $\pm$ 0
<b>Saturated (g)</b>	12 $\pm$ 3	15 $\pm$ 4	15 $\pm$ 4
<b>Carbohydrate (g)</b>	70 $\pm$ 9	78 $\pm$ 5	78 $\pm$ 5
<b>Protein (g)</b>	33 $\pm$ 2	35 $\pm$ 1	35 $\pm$ 1

#### 2.4.5 Oral fat tolerance tests (OFTT)

The OFTT meal composition of all study meals are reported in *Table 2.2*. Study 1 (Chapter 3) and the high fat – low carbohydrate meal of study 2 (Chapter 4) included a non-proprietary high fat meal designed specifically for this investigation and made with dairy products and flavoured with chocolate powder. The high fat – high carbohydrate meal of study 2 included an additional 80g of dextrose powder. Study 3 (Chapter 5) was also made with dairy products but flavoured with either freeze-dried strawberries (intervention) or a commercially available milkshake mix powder (placebo) (Tesco,

UK). The high fat meals were designed for participant palatability and in accordance with Oral Fat Tolerance Test expert statement guidelines which recommended 75g fat, 25g carbohydrates, 10g protein (Kolovou et al. 2011).

**Table 2.2** Meal composition of OFTTs across studies.

	<b>Study 1 &amp; 2 HFLC</b>	<b>Study 2 HFHC</b>	<b>Study 3 intervention</b>	<b>Study 3 placebo</b>
<b>Volume (ml)</b>	375	375	375	375
<b>Energy (Kcal)</b>	823	1115	831	832
<b>Fat (g)</b>	75	75	74	73
<b>Saturated (g)</b>	47	47	46	46
<b>Carbohydrate (g)</b>	22	95	32	33
<b>Fructose (g)</b>	0	0	6	0
<b>Protein (g)</b>	14	14	12	11
<b>Fibre (g)</b>	2	2	3	0.1

*HFLC, high fat – low carbohydrate; HFHC, high fat – high carbohydrate.*

#### 2.4.6 Blood sampling

Blood samples were drawn from a 20 gauge peripheral venous cannula (Braun Introcan Safety 20G Closed Catheter, Pennsylvania, USA) inserted by Alasdair O’Doherty in to a vein in the lower arm. The cannula was kept patent between blood draws with a mandrin stylet (Braun Vasofix Stylet, Pennsylvania, USA). Up to 25ml of blood was drawn at each time point. EDTA and fluoride oxalate blood collection tubes were spun immediately at 2383g for 15 minutes at 4°C, SST II blood collection tubes were stored at room temperature to clot for 30 minutes and then spun at 1992g for 10 minutes at 4°C. Plasma from EDTA and fluoride oxalate tubes, and serum from the SST II tubes were aliquoted and stored at -80°C until analysis.

## 2.5 Biochemistry analyses

The lead researcher received training on the Pentra 400 biochemistry auto-analyser (Horiba, UK) from Horiba. The lead researcher performed the calibration, quality controls and analyses of TGs, cholesterol (total and HDL-c), glucose, Apo A1, Apo B. The lead researcher performed the oxLDL analyses under the supervision of an experienced biochemist. The lead researcher performed the FOX1 assay to assess lipid hydroperoxides under the supervision of a trained biochemist. A university biochemist performed analyses on the strawberry product using the Folin-Ciocalteu assay with assistance of the lead researcher. The insulin analyses was performed by a biochemist at the local National Health Service (NHS) blood bioscience laboratory.

### 2.5.1 Triglycerides, cholesterol, apolipoprotein and glucose analyses

The ABX Pentra 400 biochemistry autoanalyser (Horiba, Montpellier, France) was used to analyse serum TG (intra assay variation for study 1, 2 and 3 was: 1.3%, 0.9% and 1.2%, respectively), total cholesterol (intra assay variation for study 1, 2 and 3 was: 0.6%, 0.5% and 0.6%, respectively), high density lipoprotein cholesterol (HDL-c) (intra assay variation for study 1, 2 and 3 was: 1.0%, 0.8% and 0.5%, respectively), plasma glucose (intra assay variation for study 1, 2 and 3 was: 1.1%, 1.6% and 0.9%, respectively) and apolipoproteins A1 (intra assay variation for study 1 was: 1.6%), and B (intra assay variation for study 1 was: 2.0%). Calibration and quality controls were performed prior to use in accordance with manufacturer's guidelines and samples were measured in duplicate. The machine was re-calibrated when any analyte control did not fall within the accepted range. Low Density Lipoprotein (LDL-c) was estimated from the Friedewald equation (Friedewald et al., 1972).

### 2.5.2 Insulin analyses

Insulin was measured in single using an ultrasensitive insulin assay on a Beckman Coulter DXI analyser (Beckman Coulter inc, USA). This testing was performed by the lead biochemist at the local National Health Service (NHS) blood biosciences laboratory. The analyser is used for clinical assessment and research trials across the hospital. The analyser is assessed as part of national external quality assessment scheme (EQAS), total imprecision was <10% for low to medium range control and <12% for high controls which is comparable to other UK centres and considered to be an acceptable level of imprecision for insulin by the NHS trust.

### 2.5.3 Lipid oxidation analyses

Serum oxLDL was determined by using an enzyme-linked immunosorbent assay (ELISA) performed by Alasdair O'Doherty under the guidance of a University biochemist, in accordance with the manufacturer's guidelines (Merckodia Inc, Upsala, Sweden), each sample was measured in duplicate. The intra assay variation was 3.3% and inter assay variation was 3.8%. Serum lipid peroxidation was estimated by using the ferrous oxidation in xylenol orange (FOX1) assay in line with established methods (Wolff, 1994). This assay was performed by Alasdair O'Doherty under the guidance of a University biochemist. The FOX1 reagent was made by combining the following: 100  $\mu\text{M.L}^{-1}$  Xylenol orange, 250  $\mu\text{M.L}^{-1}$  Ammonium ferrous sulphate, 100  $\text{mM.L}^{-1}$  Sorbitol and 25  $\text{mM.L}^{-1}$  of sulphuric acid. The FOX1 reagent was then stored at room temperature (21°C) in the dark. The standards were made from the following dilutions of hydrogen peroxide; 0.00, 0.31, 0.63, 1.25, 2.50 and 5.00  $\mu\text{M.L}^{-1}$ . The samples were left to thaw on ice in dark conditions. Twenty microlitres of the sample or standard was mixed with 180  $\mu\text{L}$  of the FOX1 reagent, vortexed, and then pipetted in to the 96 well

plate. The 96 well plate containing the vortexed samples and standards was left to incubate for 30 minutes at room temperature (21°C) in the dark. The absorbance of the samples and standards were read at 560 nm and the concentrations of the samples were determined using the standard curve. The standard curve was plotted using the known micromolar concentrations of the standards, stated above. The intra-assay variation was 2.1%.

#### 2.5.4 Folin-Ciocalteu assay

The Folin-Ciocalteu assay was performed on the freeze dried strawberry product and on the placebo product to provide an estimate of antioxidant capacity. The assay was performed by a University biochemist in keeping with established methods but using epicatechin equivalents in place of gallic acid equivalents (Singleton and Rossi, 1965). The strawberry/placebo product was mixed with 100% dimethyl sulfoxide to make a 50 mg·mL<sup>-1</sup> sample concentration. Then 15 µL of this sample, 170 µL double-distilled water, 12 µL Folin-Ciocalteu reagent and 30 µL sodium carbonate solution (concentration 200 g·L<sup>-1</sup>) was added to each well of a 96 well plate. This was incubated in the dark for 1 hour at 21°C and then 73 µL double-distilled water was added to each well. Absorbance was then measured at 765 nm.

## Chapter 3: The repeatability of the abbreviated (4-hour) Oral Fat Tolerance Test and influence of prior acute aerobic exercise

This study was published in the European Journal of Nutrition on 14<sup>th</sup> October 2016:

**O'Doherty AF**, Sathyapalan T, Rigby AS, Ingle L, Carroll S (2016) The repeatability of the abbreviated (4-h) Oral Fat Tolerance Test and influence of prior acute aerobic exercise. *Eur J Nutr*. Epublished 14.10.2016

<https://link.springer.com/article/10.1007%2Fs00394-016-1320-z>

### 3.1 Introduction

The Oral Fat Tolerance Test is used to assess the capacity to adapt postprandial metabolic processes after a predefined oral fat load and evaluate cardio-metabolic health (Alipour et al., 2008a, Ryan et al., 2013, Maraki et al., 2011, Madec et al., 2011, Kolovou et al., 2011). After oral fat consumption (>15g), TG levels rise in the blood, typically peaking at 3-4 hours and returning to baseline 6-8 hours later (Pirillo et al., 2014). These rises are exacerbated in those with cardiovascular and metabolic disorders and are associated with progression of atherosclerosis (Pirillo et al., 2014, Alipour et al., 2008a, Boren et al., 2014). Since humans spend most of the day in the postprandial state, OFTT may reveal cardio-metabolic dysfunction not detected by traditional fasting measures (Pirillo et al., 2014, Krug et al., 2012, Weintraub et al., 1996).

At present, the OFTT is not widely used in a clinical setting to assess cardio-metabolic function. One reason for this could be the time demands of OFTT (Weiss et al., 2008) which typically require postprandial measurements to be taken every 1 or 2 hours for 5-8 hours (Zhang et al., 2004, Plaisance et al., 2008, Pfeiffer et al., 2005, Petridou et al., 2004, Katsanos and Moffatt, 2004, Clegg et al., 2007). However, recently an abbreviated OFTT (lasting 4-hours) has been developed and validated (Weiss et al., 2008, Maraki et al., 2011). This test would reduce the time constraints, improve the

practicality of OFTT (Weiss et al., 2008) and has been recommended by an expert consensus as clinically relevant and the most representative time to measure postprandial TG responses following an OFTT (Kolovou et al., 2011, Mihas et al., 2011). Furthermore, the OFTT meal constitution (fat and carbohydrate content) is inconsistent across research studies that have used an OFTT to induce postprandial lipaemia (see *Table 1.1*). Expert panel guidelines have also recommended OFTT meals to contain approximately; 75g fat, 25g carbohydrate, 10g protein (Kolovou et al., 2011). Understanding the repeatability of an OFTT is central to implementing this test in clinical and research environments. Postprandial TG after an OFTT is reported to have high repeatability in healthy (Gill et al., 2005, Weiss et al., 2008, Ryan et al., 2013) and overweight or obese adult participants (Weiss et al., 2008, Ryan et al., 2013). However, the statistical measures of agreement employed in these studies could be conceived as misleading with respect to attaining clinically meaningful measurement for repeatability, as discussed by Bland & Altman (Bland and Altman, 1999). Therefore, an assessment of the repeatability of the 4-hour OFTT with the meal composition meeting recommended guidelines and agreement assessed using Bland-Altman analyses is required.

Due to the relationship between elevated postprandial TG and increased cardio-metabolic risk, interventions to acutely reduce postprandial hyperlipidaemia have been investigated. Acute aerobic exercise performed within 24 hours of OFTT ingestion has often been shown to be an effective intervention in reducing postprandial TG (for reviews see; (Maraki and Sidossis, 2013, Freese et al., 2014, Plaisance and Fisher, 2014)). An under-researched area, where conflicting data exist, relates to exercise performed shortly before an OFTT to lower postprandial stresses (Plaisance et al., 2008, Ferreira et al., 2011, Pfeiffer et al., 2005, Petridou et al., 2004, Cox-York et al., 2013, Katsanos and Moffatt, 2004, Clegg et al., 2007). Exercise performed shortly before an

OFTT either lowers (Plaisance et al., 2008, Ferreira et al., 2011, Katsanos and Moffatt, 2004) or has no effect on reducing postprandial lipaemia (Pfeiffer et al., 2005, Petridou et al., 2004, Cox-York et al., 2013, Clegg et al., 2007). To the author's knowledge, the repeatability of postprandial responses to OFTT with prior aerobic exercise (performed at any time point) has not been investigated. Due to the small sample sizes in the above cited studies, if exercise has a highly variable within person effect, this could account for the inconsistencies in the literature. Therefore, understanding the variability of postprandial TG after OFTT with prior exercise is paramount for study design (sample size calculation) and interpretation of these data. An assessment of the repeatability of postprandial TG to an abbreviated OFTT after acute exercise is required to address this issue.

The aim of this study was to investigate the repeatability of postprandial TG after an abbreviated 4-hour OFTT with and without prior aerobic exercise in apparently healthy adult males.

## 3.2 Methods

### 3.2.1 Participants

Apparently healthy adult males volunteered for this study that adhered to the inclusion and exclusion criteria within General Methods (Chapter 2.1). This study was conducted according to the declaration of Helsinki and approved by the Department of Sport, Health and Exercise Science Ethics Committee, University of Hull. Written informed consent was given by all participants prior to commencing in the study.



### 3.2.2 Study Design

This randomised crossover study investigated the repeatability of acute postprandial lipaemic responses (serum TG concentrations) under two experimental conditions; 1. OFTT rest condition, 2. OFTT undertaken immediately after continuous aerobic exercise. Participants attended the research laboratory before 10:00am on five separate occasions; one screening visit, two visits under the rest condition and two visits under the exercise condition. Each experimental visit was randomised and separated by at least 72 hours. All tests were completed within 8 weeks of the screening visit.

### 3.2.3 Screening Visit

Baseline stature (Harpenden Stadiometer, Holtain Limited, Crymych Pembrokeshire), body mass (Seca 635 platform scales, Hamburg, Germany), waist and hip circumferences (Seca 201 ergonomic circumference measuring tape, Hamburg, Germany) and estimated body fat percentage (BF900 Maltron Body Composition Analyser, Essex, UK), detailed in the General Methods (Chapter 2.3) were recorded. Participants then underwent a 2-hour oral glucose tolerance test (Chapter 2.3.4) (OGTT) and a cardiopulmonary exercise test (Chapter 2.3.5) (CPET).

### 3.2.4 Visits 1-4

An evening meal (detailed in Chapter 2.4.4) was provided by the research team and consumed by the participant at home (unsupervised) on the evening before each laboratory visit. Participants fasted overnight (>12 hours) and attended the laboratory the following morning. Baseline measures taken on the screening visit (detailed above) were repeated. Participants performed standardised continuous moderate intensity

aerobic exercise (Chapter 2.4.2), if randomised to the exercise condition, and consumed an OFTT meal (Chapter 2.4.5) immediately afterwards. Blood samples were taken at 1, 2, 3 and 4-hour time points after the OFTT meal was consumed. Serum TG, total cholesterol, HDL-c, apolipoprotein A1, apolipoprotein B, glucose (ABX Pentra, Horiba, France) and insulin (Beckman Coulter DXI, USA) were analysed (Chapter 2.5). LDL-c was estimated using the Friedwald equation (Friedewald et al., 1972). An abbreviated 4-hour time period to assess the postprandial response to OFTT was selected following the initial work of Weiss and colleagues (Weiss et al., 2008) which has since been validated by Maraki and colleagues (Maraki et al., 2011).

### 3.2.5 Outcome Measures

The primary outcome for this study was postprandial TG AUC following the 4-hour OFTT. Secondary outcome measures were AUC for Apolipoprotein B, glucose and Insulin after 4-hour OFTT.

### 3.2.6 Statistical Analysis

Normal (Gaussian) distribution of data was verified using the Shapiro-Wilk test, tests for skewness and kurtosis of distributions and visual inspection of histogram charts. Non-normally distributed data were transformed and analysed using parametric statistics where possible, and non-parametric analyses was performed when after transformation criteria for normal distribution, stated above, was not met. Data are presented as mean and standard deviation (SD) for parametric data, and non-normally distributed data are presented as median and quartiles 1 and 3 (Q1, Q3). AUC was determined by the trapezoidal method (Matthews et al., 1990). To determine agreement

between repeated measures Bland-Altman plots were used (Bland and Altman, 1999), 95% limits of agreement were estimated for parametric analyses. TG data was non-normally distributed and could not be transformed, as such, both the parametric and non-parametric approaches to the Bland-Altman plot are presented. Bland and Altman state; “if there are one or more extreme discrepancies between the methods a nonparametric approach may be felt preferable” (Bland and Altman, 1999). As such, non-parametric Bland-Altman methods with predefined arbitrary limits of agreement set to assess how many data points fell within these arbitrary limits were used. This provides a simple and more appropriate method for the reader to interpret repeatability in non-normally distributed data. An arbitrary limit of 15% of the median TG AUC was selected because exercise interventions typically reduce postprandial TG by  $\geq 15\%$  (Maraki and Sidossis, 2013) therefore setting the upper limit of acceptable repeatability for exercise intervention studies. An arbitrary 10% limit was set in keeping with the findings of Gill and colleagues who reported a 10% variation for within-person postprandial TG responses to OFTT in men (Gill et al., 2005). Spearman’s ranked correlations and a novel statistical method proposed by Ryan and colleagues (Ryan et al., 2013), which does not assume normality, were also performed to assess variability of TG response to OFTT. Microsoft Excel (2013) and SPSS (Version 22) (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses. Whole body insulin sensitivity was estimated from relevant insulin and glucose measurements during the oral glucose tolerance test using the Matsuda index (Matsuda and DeFronzo, 1999).

A sample size of 11 male participants was proposed by Gill and colleagues to detect a 10% change ( $\alpha=0.05$  and 80% power) in TG in intervention studies incorporating OFTT (Gill et al., 2005). This sample size was selected to investigate the within-person variation prior to incorporating it in to future prospective interventional studies targeting a reduction in TG AUC after OFTT that are included within this thesis.

### 3.3 Results

Eleven apparently healthy males, median (Q1, Q3) age 30 (27, 44) years, mean (SD) body mass 78.3 (9.7) kg, and body mass index (BMI) 25.3 (3.1) kg.m<sup>-2</sup> were assessed, participant demographics are reported in *Table 3.1*. Data for one participant was excluded for the rest condition due to breach of inclusion criteria, the participant stated (at the end of their study visit) that they had consumed alcohol within 24 hours of the test. Accordingly, 10 complete datasets are reported for the rest condition. Intra-individual variation of baseline fasting TG concentration derived from the four fasting measurements (n=10) was 19.1%. All participants completed the continuous moderate intensity aerobic exercise intervention on a cycle ergometer, on two separate days, immediately before OFTT ingestion. Median (Q1, Q3) work rate at 90% AT was 70 (67, 76) W. Median (Q1, Q3) estimated energy expenditure was 250 (221, 252) kcal on the first exercise intervention and 243 (230, 269) kcal on the second exercise intervention. Median respiratory quotient during exercise at 90%AT was 0.90 (0.88, 0.92) and 0.89 (0.87, 0.91) for the first and second exercise intervention, respectively. Median heart rate was 105 (102, 109) and 104 (102, 110) beats.min<sup>-1</sup> for the first and second exercise intervention, respectively. Accordingly, the metabolic responses were consistent between the first and second acute exercise interventions.

**Table 3.1** Baseline demographics (mean (SD))

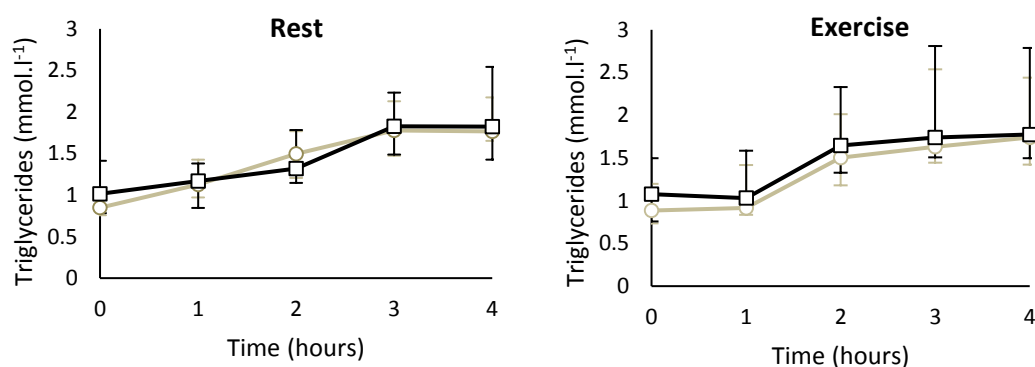
Baseline participant demographics	
Number of participants	11 male
Age (years)*	30.0 (27, 44)
Weight (kg)	78.3 (9.7)
BMI (kg.m <sup>-2</sup> )	25.3 (3.1)
Waist hip circumferences ratio	0.86 (0.06)
Body fat content (%)*	21.0 (5.0)
$\dot{V}O_2$ peak (ml.kg <sup>-1</sup> .min <sup>-1</sup> )*	32.5 (32.0, 37.4)
AT (ml.kg <sup>-1</sup> .min <sup>-1</sup> )*	15.7 (14.8, 18.5)
Matsuda Index	10.1 (4.7)
HOMA IR	0.97 (0.41)
Triglyceride (mmol.l <sup>-1</sup> )*	1.0 (0.7, 1.3)
Cholesterol (mmol.l <sup>-1</sup> )*	5.4 (4.5, 5.6)
HDL-C (mmol.l <sup>-1</sup> )*	1.4 (1.3, 1.5)
LDL-C (mmol.l <sup>-1</sup> )*	3.4 (2.9, 3.8)
Apo B:Apo A1	0.73 (0.08)

\*denotes Median (Q1, Q3),

Blood measures are fasting measures, excluding the Matsuda index

### 3.3.1 Serum triglyceride response to OFTT

Figure 3.1 Shows the median and Quartiles 1 and 3 for TG responses at each time point during OFTT. Median TG showed incremental increases during the resting and post-exercise OFTT attaining a peak concentration at 3 to 4 hours.



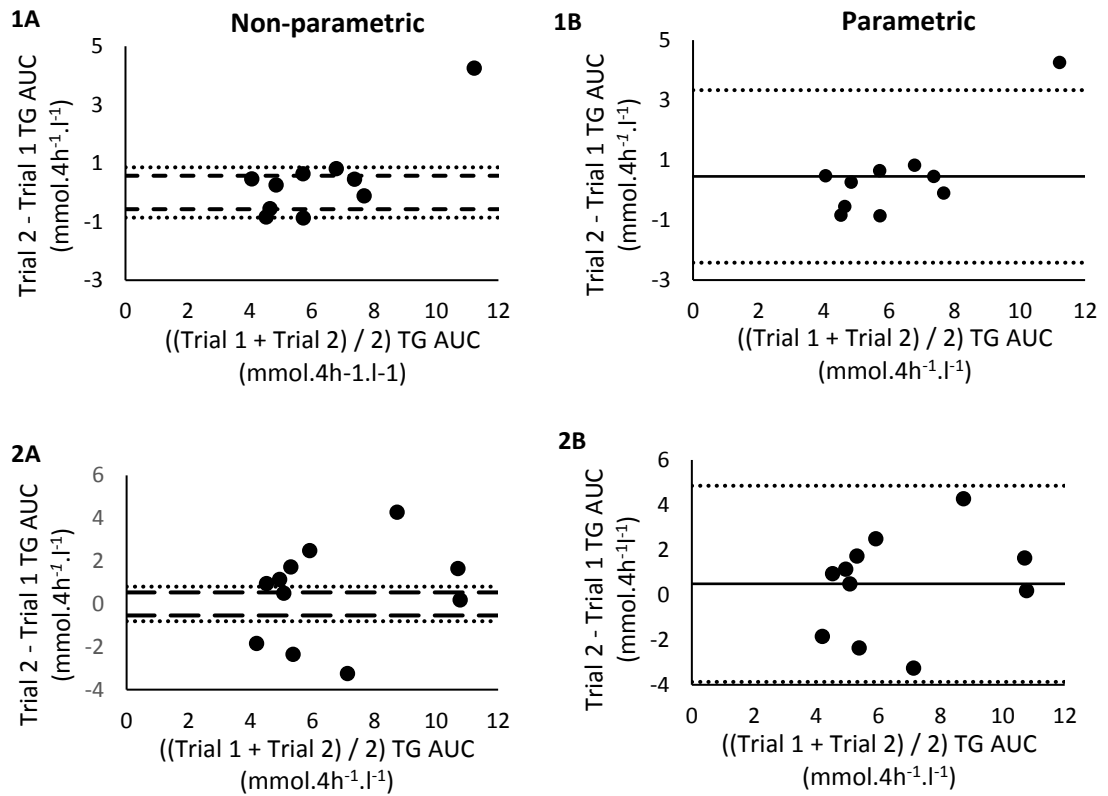
**Figure 3.1** Median (Q1, Q3) TG responses to OFTT during each trial. Left Panel: Circles (light grey line) denote the first OFTT and squares (black line) the second OFTT under the rest condition (n=10). Right panel: Circles (light grey line) denote the first OFTT and squares (black line) denote the second OFTT under the exercise condition (n=11).

### 3.3.1.1 *Non-parametric Bland-Altman analysis*

Figure 3.2 shows non-parametric Bland-Altman plots for TG AUC for the repeated tests under each trial condition; rest (panel 1A) and exercise condition (panel 2A). To assess repeatability, limits of agreement were predefined at  $\pm 10\%$  and  $\pm 15\%$  of the median score of all tests. For the rest condition, the limits of agreement for  $\pm 10\%$  of the median were  $-0.57$  to  $0.57$   $\text{mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$  and for  $\pm 15\%$  of the median were  $-0.86$  to  $0.86$   $\text{mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$ . For the exercise condition, the limits of agreement for  $\pm 10\%$  of the median were  $-0.54$  to  $0.54$   $\text{mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$  and for  $\pm 15\%$  of the median were  $-0.81$  to  $0.81$   $\text{mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$ . Five of 10 data points fell within the 10% limits of agreement for the rest condition and two of the 11 data points for the exercise condition. Nine of 10 data points fell within the 15% limits of agreement for the rest condition and two of 11 data points for the exercise condition.

### 3.3.1.2 *Parametric Bland-Altman analysis*

Parametric Bland Altman plots for the rest and exercise condition are shown in panels 2A and 2B, respectively. The mean bias for the rest condition was  $0.46$  (95% CI:  $-0.59$  to  $1.51$ ) and 95% LOA were  $-2.42$  (95% CI:  $-3.89$  to  $-0.95$ ) to  $3.33$  (95% CI:  $1.87$  to  $4.80$ )  $\text{mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$ . The mean bias for the exercise condition was  $0.49$  (95% CI:  $-1.01$  to  $1.99$ ) and 95% LOA were  $-3.87$  (95% CI:  $-6.89$  to  $-0.85$ ) to  $4.85$  (95% CI:  $1.83$  to  $7.87$ )  $\text{mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$ .



**Figure 3.2** Non-parametric Bland Altman plots of postprandial TG AUC after rest condition (panel 1A, n=10) and exercise condition (panel 2A, n=11). Dashed lines denote  $\pm 10\%$  and dotted lines denote  $\pm 15\%$  of the median TG area under curve. Parametric Bland Altman plots of TG AUC after rest condition (panel 1B, n=10) and exercise condition (panel 2B, n=11). Dotted lines denote 95% limits of agreement (LOA) and black solid lines denote the mean bias for each condition.

### 3.3.1.3 Spearman's ranked correlations

The Spearman's ranked correlation between AUC of trial 1 and trial 2 of the rest and exercise conditions can be observed in *Table 3.2a* and *3.2b*. Spearman's ranked correlations were  $\rho = 0.90$  and  $\rho = 0.42$  for the rest and exercise conditions, respectively. Five participants had the same rank on each test (rank difference = 0) in the rest trial leading to a high ranked correlation coefficient of 0.90, whereas none of the participants had the same rank for the exercise condition.

**Table 3.2a** Spearman's ranked correlation for the rest condition

	Rest 1	Rest 2	Rank 1	Rank 2	Difference	Difference <sup>2</sup>
R09	3.83	4.30	1	2	-1	1
R06	4.72	4.98	2	4	-2	4
R08	4.92	4.37	3	3	0	0
R11	4.95	4.11	4	1	3	9
R05	5.39	6.04	5	6	-1	1
R10	6.16	5.30	6	5	1	1
R03	6.37	7.19	7	7	0	0
R07	7.14	7.60	8	8	0	0
R02	7.73	7.63	9	9	0	0
R01	9.09	13.35	10	10	0	0
					Sum	16
					Sum*6	96
					(Sum*6)/n(n2-1)	0.10
					Rank Correlation (rho)	0.90

**Table 3.2b** Spearman's ranked correlation for the exercise condition

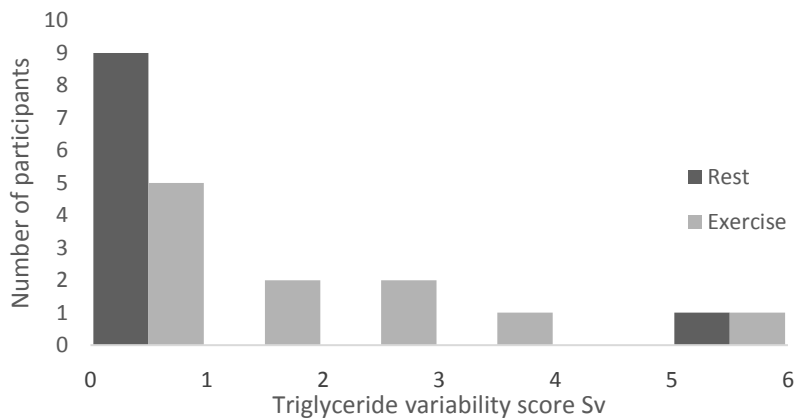
	Exercise 1	Exercise 2	Rank 1	Rank 2	Difference	Difference <sup>2</sup>
R11	4.05	5.00	1	3	-2	4
R06	4.38	5.51	2	6	-4	16
R05	4.45	6.17	3	7	-4	16
R03	4.67	7.15	4	8	-4	16
R04	4.83	5.32	5	4	1	1
R08	5.12	3.27	6	1	5	25
R09	6.56	4.20	7	2	5	25
R10	6.61	10.88	8	10	-2	4
R02	8.76	5.51	9	5	4	16
R01	9.88	11.52	10	11	-1	1
R07	10.68	10.86	11	9	2	4
					Sum	128
					Sum*6	768
					(Sum*6)/n(n2-1)	0.58
					Rank Correlation (rho)	0.42

### 3.3.1.4 A novel assessment of variation

A variation score was calculated to assess repeatability, the variation of this score across the groups can be observed in *Figure 3.3*. The closer the score is to 0, the smaller the



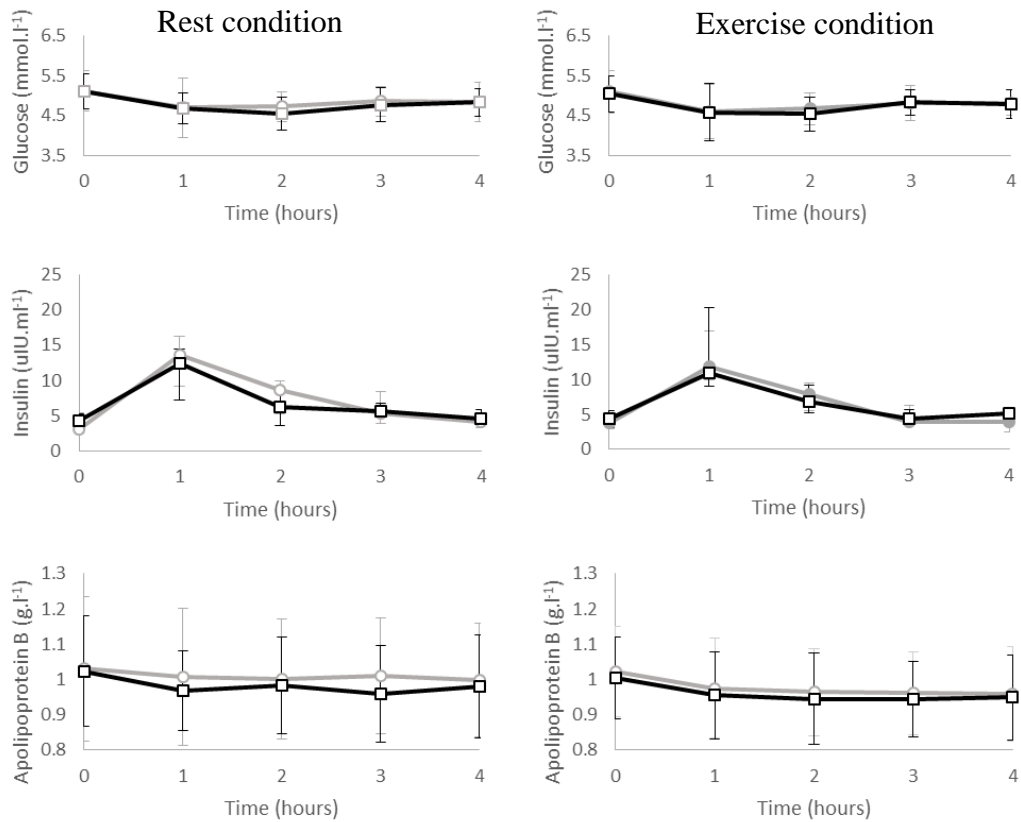
variation of the measure. Nine of the 10 data points scored <1 for the rest condition compared to 5 of 11 data points for the exercise condition.



**Figure 3.3** Variation score for individual participants for the rest condition (dark grey, n=10) and the exercise condition (light grey, n=11).

### 3.3.2 Glucose, Insulin and Apolipoprotein B responses to OFTT

*Figure 3.4* shows the 4-hour responses of glucose, insulin and apolipoprotein B to the OFTT. The limits of agreement (LOA) and mean bias for apolipoprotein B, glucose, and insulin are reported in *Table 3.3*. Insulin AUC was not reported for one participant in the rest condition due to haemolysis of a serum sample at the 1 hour time point, insulin AUC data are reported on 9 participants.



**Figure 3.4** Mean (SD) glucose responses (top panels), median (Q1, Q3) insulin responses (middle panels) and mean (SD) apolipoprotein B responses (bottom panels) to OFTT during each trial. Left Panels: Circles (light grey line) denote the first OFTT and squares (black line) the second OFTT during the rest condition (glucose and apolipoprotein, n=10; insulin, n=9). Right panels: Circles (light grey line) denote the first OFTT and squares (black line) denote the second OFTT under the exercise condition (n=11).

**Table 3.3** Repeatability of glucose, insulin and apolipoprotein 4-hour AUC after Oral Fat Tolerance Test

Substrate	Grand Mean	SD	Mean Bias	Lower 95% LOA	Upper 95% LOA
<i>Glucose (mmol.4h<sup>-1</sup>.l<sup>-1</sup>)</i>					
OFTT	<b>18.88</b>	<b>1.13</b>	<b>-0.26</b>	<b>-2.40</b>	<b>1.89</b>
OFTT + Exercise	<b>19.10</b>	<b>1.35</b>	<b>-0.07</b>	<b>-2.50</b>	<b>2.36</b>
<i>Insulin (uIU.4h<sup>-1</sup>.ml<sup>-1</sup>)*</i>					
OFTT**	<b>33.34</b>	<b>16.47</b>	<b>0.91</b>	<b>0.70</b>	<b>1.18</b>
OFTT + Exercise	<b>31.02</b>	<b>12.10</b>	<b>1.03</b>	<b>0.62</b>	<b>1.73</b>
<i>Apo B (g.4h<sup>-1</sup>.l<sup>-1</sup>)</i>					
OFTT	<b>3.97</b>	<b>0.59</b>	<b>-0.12</b>	<b>-0.85</b>	<b>0.60</b>
OFTT + Exercise	<b>3.86</b>	<b>0.47</b>	<b>-0.07</b>	<b>-0.46</b>	<b>0.33</b>

*SD is Standard Deviation; 95% LOA are the 95% limits of agreement; \*Insulin data were log transformed therefore geometric mean and SD are reported and mean bias with 95% LOA are reported as a ratio; \*\*Insulin AUC for OFTT n=9.*

### 3.4 Discussion

The results from the present study demonstrate that 4-hour TG responses to OFTT in men are repeatable, however, when aerobic exercise is performed immediately before OFTT the repeatability is poor. Secondary findings demonstrate that repeatability of glucose was good, with wider limits of agreement for both insulin and apolipoprotein B responses to OFTT (limits of agreement within  $\pm 11\%$ ,  $-21\%$  to  $27\%$  and  $\pm 18\%$  of the mean, respectively). Limits of agreement for glucose and apolipoprotein B after OFTT with prior exercise were good (within  $13\%$  and  $10\%$  of the mean) but insulin showed very poor repeatability (LOA from  $-41\%$  to  $+70\%$ ).

#### 3.4.1 Repeatability of TG AUC in the resting condition

The within person variation of a previously validated abbreviated 4-hour OFTT was investigated (Weiss et al., 2008, Maraki et al., 2011). The repeatability of TG responses to OFTT with measurements taken for 5 to 8 hours post ingestion have been demonstrated previously, however, absolute measures of limits of agreement have not been reported (Ryan et al., 2013, Gill et al., 2005, Weiss et al., 2008, Brown et al., 1992). The author identified, using non-parametric Bland-Altman analyses, that 90% of the data points fell within  $\pm 0.86 \text{ mmol.l}^{-1}$  equating to  $\pm 15\%$  of the median. This value could be considered as a clinically meaningful change for intervention studies utilising 4-hour OFTT. Spearman's ranked correlations also highlight the strong relationship between the two rest trials ( $\rho = 0.90$ ) and weaker relationship between the two exercise trials ( $\rho = 0.42$ ). The author tried to reduce variation by controlling the composition of the evening meal prior to the OFTT as this has been reported to alter the TG response (Robertson et al., 2002). The author also employed a recently proposed (Ryan et al., 2013) assessment of variability in TG responses to OFTT and identified that 9 of 10

participants had very low variation and one participant having very large variation between the two OFTTs. In keeping with the literature, higher fasting and peak TG responses are prone to greater variation (Ryan et al., 2013). Such variation is not uncommon, Ryan and colleagues (Ryan et al., 2013) reported variation in 18% of their population and associate this variation or “deviation from the norm” (Ryan et al., 2013) with phenotypic and genotypic characteristics. The proposed existence of a variable phenotype supports the statistical methods that were employed in this study to assess repeatability as these ‘outliers’ cause a non-normal distribution which would over-estimate limits of agreement using parametric or log transformed Bland-Altman plots particularly with the small sample size.

#### 3.4.2 Repeatability of TG AUC in the exercise condition

Exercise interventions prior to OFTT have been shown to be effective in reducing postprandial TG responses. However, to the author’s knowledge the repeatability of this effect has not been investigated prior to this study. This study demonstrates that exercise performed immediately prior to OFTT ingestion provokes highly variable postprandial TG responses. This variation could explain the inconsistency in the findings of exercise intervention studies where immediate prior exercise has been shown to reduce (Plaisance et al., 2008, Katsanos and Moffatt, 2004, Ferreira et al., 2011) or have no effect (Clegg et al., 2007, Pfeiffer et al., 2005, Cox-York et al., 2013, Petridou et al., 2004) on the postprandial TG response. Caution should therefore be taken with regards to interpretation of studies employing aerobic exercise interventions immediately prior to OFTT. Implications of this finding for clinical practice would be limiting moderate or vigorous physical activity, such as a long walk to the hospital or clinic, immediately prior to OFTT.

### 3.4.3 Parametric repeatability analysis of TG AUC

Agreement of these data was assessed by using parametric Bland-Altman analyses, which demonstrated poor agreement with both rest and exercise trial conditions (Panel 1B and 2B, *Figure 3.2*). The assumptions of the parametric Bland-Altman approach are that data are normally distributed, that is 95% of data falls within two Standard Deviations. Clearly when data are non-normally distributed, as with this dataset, two standard deviations will not reflect 95% of these data and therefore the limits of agreement calculated from two standard deviations have no relevance to these data. This is particularly apparent in panel 1B of *Figure 3.2*, where the apparent ‘outlier’ markedly widens and therefore over-exaggerates the limits of agreement. The failure of this dataset to meet the fundamental assumptions of parametric analysis supports the use of the non-parametric methods selected and used to formulate the conclusions.

### 3.4.4 Carbohydrate content of OFTT meals

The OFTT meal composition for this study had a lower carbohydrate content than some exercise intervention studies (Hurren et al., 2011, Ferreira et al., 2011, Pfeiffer et al., 2005, Petridou et al., 2004, Cox-York et al., 2013) but similar to others (Katsanos and Moffatt, 2004, Plaisance et al., 2008, Clegg et al., 2007). Carbohydrate and protein enable lipid absorption and the quantities that were used in the present study were similar to the OFTT proposed in an expert panel statement that had considered lipid absorption in the design of the meal (Kolovou et al., 2011). Carbohydrate ingestion stimulates increased insulin secretion which suppresses fatty acid oxidation in the liver and upregulates TG removal from plasma in to adipose and muscle tissue (Dimitriadis et al., 2011). Increased insulin as a result of a high fat (80g) – high carbohydrate (100g) meal evoked a lower postprandial TG AUC compared with a high fat (80g) – low

carbohydrate meal (20g) in healthy participants (Kriketos et al., 2003). Acute exercise is associated with improved whole body insulin sensitivity for up to 48 hours (Cartee, 2015). Therefore, the carbohydrate content of OFTT meals is an important consideration for acute exercise intervention studies incorporating OFTT but to the author's knowledge this has not been investigated.

#### 3.4.5 Baseline TG and Apolipoprotein, glucose and insulin AUC responses

The variation in baseline TG across the 4 testing days was consistent with literature on biological variation of TG (Smith et al., 1993). The limits of agreement for blood glucose suggested good repeatability as has been previously reported in OFTT (Gill et al., 2005). The relatively low carbohydrate content of the meal led to small changes in glucose concentrations from baseline to 4-hours. The repeatability of insulin AUC in the present study was poor. These measurements indicated that insulin concentrations were highest at the 1 hour time point during OFTT and returning to baseline concentrations at the 2, 3 and 4-hour time points. The apparent poor agreement in insulin AUC is most likely due to insufficient measurements taken around the peak circulating insulin time point, rather than the variability of the insulin response. Therefore, the hourly blood sampling time points of this study may not be appropriate to assess the postprandial insulin responses to OFTT. Apolipoprotein B showed good limits of agreement for the exercise condition with wider limits of agreement for the rest condition. Total apolipoprotein B responses to OFTT appears to be less susceptible to acute changes compared to TG responses, consistent with previous findings among healthy, obese and hyperlipidaemic participants (Otokozawa et al., 2009). The within person variability of apolipoprotein B to OFTT should be considered meaningful for future studies assessing this measure.

### 3.4.6 Strength and limitations

The strengths of this study include the robust study design which allowed investigation in to the repeatability of OFTT under two conditions. The intensity of exercise was rigorously controlled, previous OFTT exercise intervention studies have selected exercise intensities as a percentage of  $\dot{V}O_{2peak}$  which does not necessarily control for exercise intensity domains. Furthermore, controlling food ingestion on the evening before the OFTT visits was attempted by providing participants with a standardized meal and instructing them to consume this meal as their only food intake prior to the OFTT. Limitations include the small sample size enrolled in the study, however, this was selected based on sample size recommendation for interventional studies within this area of research. Additionally, a liquid meal rather than solid meal was investigated and therefore the ecological validity of the test could be challenged. To reduce the confounding effect of exercise other than that prescribed in the protocol, participants were asked not to exercise for 24 hours before OFTT. This was in accordance with the findings of Zhang and colleagues (Zhang et al., 2004) where only exercise conducted 12 hours before OFTT and not 24 hours before OFTT reduced postprandial TG. However, other protocols required participants to refrain from exercise for up to 3 days before OFTT to remove the effect of prior exercise (Gill et al., 2005, Plaisance et al., 2008, Cox-York et al., 2013). Therefore, a confounding effect of exercise performed between 24 and 72 hours before each OFTT cannot be ruled out.

### 3.4.7 Conclusions

In conclusion, these data indicate that the postprandial TG response to the abbreviated 4-hour OFTT in men is repeatable with relevant and clinically meaningful statistical evaluation of variability. However, acute aerobic exercise performed immediately prior

to an OFTT provokes highly variable within person responses. Interpretation of data from studies investigating the effects of immediate prior acute exercise should be undertaken with caution. Future studies should investigate the repeatability of TG responses to OFTT ingestion with exercise interventions performed 8 to 24 hours before meal ingestion.



## Chapter 4: Effects of acute exercise on postprandial triglyceride responses to different lipaemic challenges in adult offspring of patients with type 2 diabetes: A randomised feasibility study

This study was accepted as an oral presentation and poster presentation at the Lipidology and cardiovascular risk management conference, Lipids, Metabolism and Vascular Risk Section. The Royal Society of Medicine.

**O'Doherty AF**, Sathyapalan T, Ingle L, Carroll S. Carbohydrate content and prior acute exercise alter postprandial lipaemic responses to high fat meals in adult male offspring of type 2 diabetics. Lipidology and cardiovascular risk management. Royal Society of Medicine. 2016

### 4.1 Introduction

The risk of developing type 2 diabetes (T2D) is increased 3.6 fold in adult offspring of patients with T2D (OT2D) (Hariri et al., 2006). Early dysregulation in glucose and/or lipid metabolism has been reported in young apparently healthy OT2D (normoglycaemic, normolipidaemic) (Eriksson et al., 1989, Henninger et al., 2014, Kriketos et al., 2004). Healthy, non-obese OT2D had raised fasting insulin and TG concentrations (Henninger et al., 2014), reduced insulin sensitivity (Kriketos et al., 2004), greater adipocyte dysfunction (Henninger et al., 2014) and lower mitochondrial activity (Petersen et al., 2004) compared to matched controls. Furthermore, OT2D with normal fasting TGs exhibit abnormal postprandial TG responses to high fat (51g) mixed meals compared to healthy matched controls (Axelsen et al., 1999). Both familial inheritance and environmental factors contribute to these risk factors which are associated with T2D and CVD (Murea et al., 2012). Additionally, conventional obesity related predictors of T2D, such as increased waist circumference and waist to hip circumferences ratio, have reduced predictive capacity in OT2D (Jafari-Koshki et al.,

2016). Other assessments to understand risk factors and underlying mechanisms of developing T2D and CVD should therefore be considered OT2D.

Measuring postprandial responses to an OFTT identifies impaired lipid handling in healthy OT2D (Axelsen et al., 1999). Mechanistic studies support that muscle insulin resistance plays a key role in increased postprandial TG synthesis after high carbohydrate mixed meals in OT2D and insulin resistant lean men (Rabøl et al., 2011, Petersen et al., 2007). The key mechanisms identified in impaired fat metabolism include dysregulation in energy storage at the muscle and increased de novo lipogenesis (DNL) within the liver (Rabøl et al., 2011, Petersen et al., 2007). The role of insulin and insulin sensitivity in postprandial TG metabolism can also be assessed with the use of OFTTs by manipulating endogenous insulin production by the incorporation of either a high or low carbohydrate content within the OFTTs. Reduced postprandial TG responses to high fat – high carbohydrate (HFHC) compared to a high fat – low carbohydrate (HFLC) meals have been observed in healthy insulin sensitive controls, but not in OT2D (who were more insulin resistant) (Kriketos et al., 2005). The reduced postprandial TG in the healthy controls after the HFHC OFTT meal appears to be insulin mediated, driven by high carbohydrate. This has been observed elsewhere in healthy groups (Cohen and Berger, 1990, Kriketos et al., 2003) and in insulin sensitive type 1 diabetic participants following a bolus of insulin during the postprandial period (Campbell et al., 2017). The authors proposed that a similar response was not observed in OT2D due to decreased insulin sensitivity (Kriketos et al., 2005). This is important because metabolic dysregulation in OT2D leads to raised postprandial TG which is associated with increased risk of CVD (Pirillo et al., 2014).

Exercise acutely improves whole body insulin sensitivity (Rohling et al., 2016) and reduces postprandial lipaemia (Gill et al., 2002, Maraki and Sidossis, 2013, Plaisance

and Fisher, 2014). This effect is transient, and lasts approximately 24 hours after exercise (Plaisance and Fisher, 2014). Despite an expert panel statement recommending low carbohydrate content (25g) in OFTTs (Kolovou et al., 2011), the carbohydrate content of OFTTs in exercise intervention studies is often in excess of 50g (Gill et al., 2002, Maraki et al., 2010), see *Table 1.1*. Therefore, exercise induced improvements in insulin sensitivity may mediate reductions in postprandial TG after OFTTs. It is important to understand whether this mechanism exists because this could inform preventative guidelines in insulin resistant populations and those at risk of cardio-metabolic disease, such as OT2D. However, the relationship between acute, moderate intensity, exercise mediated increases in insulin sensitivity and reduced postprandial TG has been discounted previously (Gill et al., 2002). Conversely, a more recent mechanistic study using MRS identified that in young, sedentary, insulin resistant males, acute exercise increased postprandial muscle glycogen synthesis and reduced hepatic TG synthesis after a high carbohydrate mixed meal (Rabol et al., 2011). As such, further investigation of this mechanism in populations at risk of cardio-metabolic disorders are required to better understand the mechanisms of exercise reducing postprandial TG responses to mixed meals. These data may support the importance of regular exercise as a preventative strategy in OT2D, provide further mechanistic insight in to metabolic dysregulation in OT2D, and provide further support for the role of improved exercise-induced muscle insulin sensitivity in reducing postprandial TG. This study will evaluate and re-emphasise that further consideration of the carbohydrate content of OFTTs designed for interventional studies is required.

To the author's knowledge evaluating acute responses to OFTTs with high and low carbohydrate content preceded by rest and exercise has not been investigated within OT2D. The aim of this feasibility study was to investigate postprandial TG responses to HFHC and HFCL meals in OT2D with and without prior acute exercise.

## 4.2 Methods

### 4.2.1 Participants

Adult males with at least one biological parent with T2D were recruited. Exclusion criteria is consistent with the General methods (Chapter 2.1) with the addition of hyperglycaemia (fasting glucose  $>7.0 \text{ mmol.l}^{-1}$  or 2-hour oral glucose tolerance test (OGTT)  $>11.1 \text{ mmol.l}^{-1}$ ). This study was conducted according to the declaration of Helsinki and approved by the Sport, Health and Exercise Science Ethics Committee at the University of Hull. Written informed consent was provided by all participants before study commencement.

### 4.2.2 Study Design

This feasibility study was a randomised, single blind, crossover study by design. There was one screening visit and four study visits with different experimental conditions. Study visits started before 10am on separate days,  $>72$  hours apart and after an overnight fast ( $>10$  hours). On two study visits participants consumed HFLC meals and on two study visits participants consumed HFHC meals (detailed in Chapter 2.4.5). Each meal (HFLC and HFHC) was preceded by rest on one visit and exercise (detailed below) on the other visit. Participants completed all experimental conditions within 8 weeks of the screening visit.

### 4.2.3 Study objectives

There were multiple study objectives for this feasibility study, these are listed below. In keeping with the recently proposed extension to the Consolidated Standards Of Reporting Trials (CONSORT) 2010 guideline (Thabane et al., 2016), a description is

provided for how each objective was planned to be addressed with relevant thresholds for successful implementation provided, where appropriate. The study objectives were:

1. To identify whether sufficient OT2D were recruited to this study from the local area within a given timeframe.

*Recruitment of all participants within 3 months of beginning the study was deemed to be successful for a future, adequately powered, single centre study.*

*The upper limit for recruitment of all participants was set at 12 months from the beginning of the study. If participants were successfully recruited within 3 to 12 months, an adequately powered study would need to be conducted across multiple centres to achieve timely study completion.*

2. To identify participant adherence to the study protocol.

*Study dropout rates will be monitored throughout the study. A dropout rate of less than 20% will be deemed favourable.*

3. To identify whether an acute exercise session influenced postprandial responses to high and low carbohydrate OFTT meal conditions and explore the role of indirect insulin sensitivity measures.

*TG AUC and iAUC will be assessed using 95% confidence intervals with an estimate of effect size used to evaluate these objectives. A large effect size (defined below in statistical analyses) will be considered a successful outcome, and where appropriate a sample size calculation for an appropriately powered study will be calculated.*

4. To identify whether carbohydrate content influenced postprandial TG responses to OFTT meals in the resting condition in OT2D.

*TG AUC and iAUC will be assessed using 95% confidence intervals with an estimate of effect size used to evaluate these objectives. A large effect size (defined below in statistical analyses) will be considered a successful outcome,*

*and where appropriate a sample size calculation for an appropriately powered study will be calculated.*

#### 4.2.4 Study recruitment

Participants were recruited through advertisements at the University of Hull, the Hull Royal Infirmary, the Hull and District Diabetes support group, and on social media. Posters were displayed on notice boards within the University of Hull and within the centre for diabetes at the Hull Royal Infirmary. An advert was placed in the University of Hull online weekly staff bulletin on three separate occasions. Adverts containing the study poster were placed on social media, targeting members of the University and local community. Finally, Alasdair O’Doherty advertised this study at the Hull and District Diabetes support group following an education session that he led on the topic of diet and exercise in people living with type 2 diabetes.

#### 4.2.5 Screening visit

Baseline stature (Harpenden Stadiometer, Holtain Limited, UK), body mass (Seca Balance Scales, Seca, Germany), waist and hip circumferences (Seca 201 ergonomic circumference measuring tape, Germany) were measured. Body fat content (percentage) was estimated using bioimpedance (BF900 Maltron Body Composition Analyser, UK). Participants performed an OGTT, detailed in chapter 2.3.4. Blood pressure (Omron M6, Omron Healthcare LTD, UK) and resting ECG measurements (GE CASE system, GE Healthcare, Germany) were recorded. Finally, participants performed a symptom-limited maximal CPET, detailed in chapter 2.3.5.

#### 4.2.6 Study visits 1-4

Participants did not exercise 24 hours before the OFTT in the rest conditions and performed 1-hour of prescribed exercise (detailed in Chapter 2) on the afternoon before OFTT in the exercise conditions. On the evening before each OFTT, participants were provided with a standardised meal to consume as their only evening nutritional intake and were instructed to consume the meal at a similar time on every evening prior to the OFTT study visit. The following morning, participants arrived at the laboratory and were provided with an OFTT (described in Chapter 2.4.5) to consume within 5 minutes, participants were blinded from the OFTT that they received. Blood was drawn at baseline and hourly for 4 hours after OFTT.

#### 4.2.7 Outcome measures

The primary objective was to determine feasibility of the methods, specifically recruitment and adherence. Other outcomes included 4-hour TG area under the curve (AUC), TG incremental AUC (iAUC), insulin AUC and glucose AUC.

#### 4.2.8 Statistical analyses

Normal distribution of data was assessed by visual inspection of histogram charts and verified using the Shapiro-Wilk test. Normally and non-normally distributed data are presented as mean (standard deviation [SD]), and median (quartiles 1 and 3 [Q1, Q3]), respectively. AUC and iAUC was determined by the trapezoidal method (Matthews et al., 1990). Repeated measures analysis of variance (ANOVA) was used to assess differences between study conditions. Mean differences with 95% confidence intervals are reported between trial conditions. Partial eta squared ( $\eta^2$ ) was used to determine

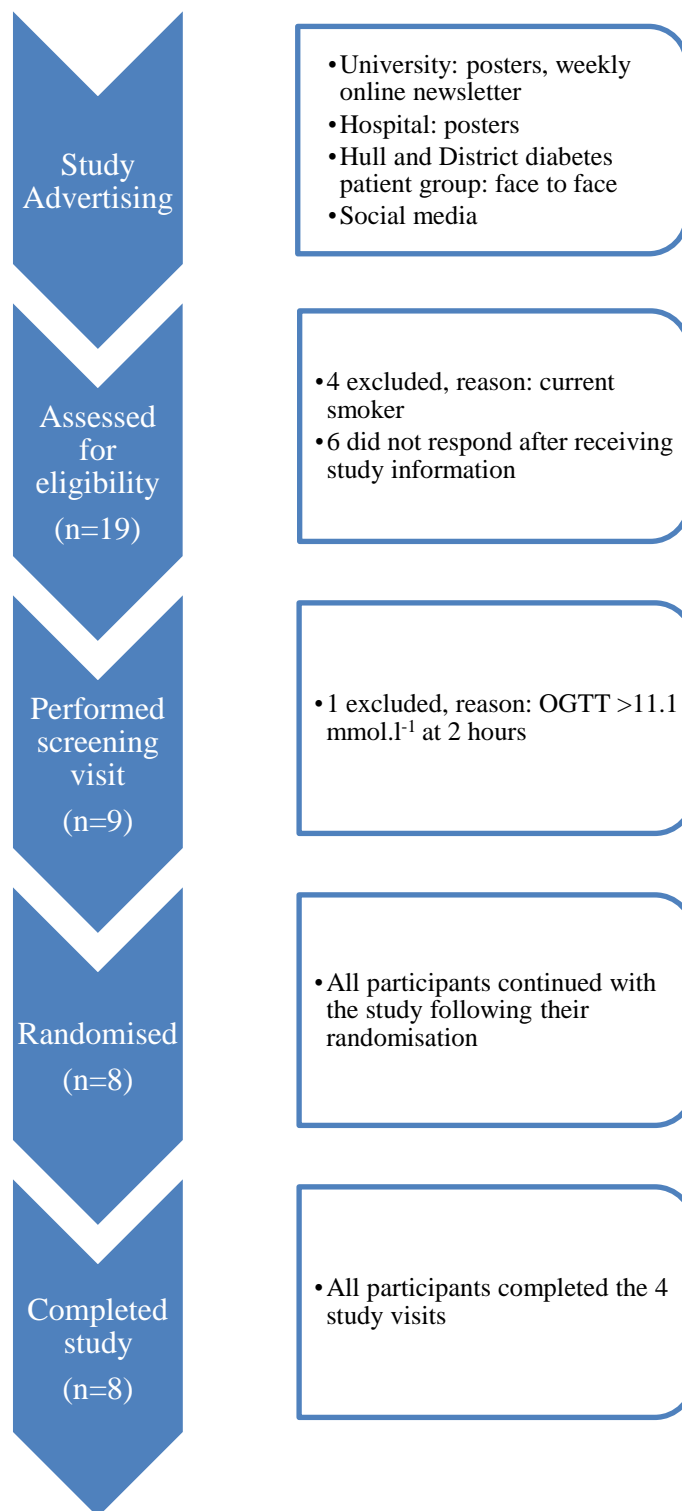
the effect size with small, medium and large effects set at 0.01, 0.06 and 0.14, respectively (Cohen, 1988). The sample size estimate (below) was performed in a healthy non-OT2D population and used only as a guide. Due to the paucity of relevant available data in OT2D for this study it was deemed that this study could not be adequately powered for hypothesis testing and it was therefore considered a feasibility study. In corroboration with guidelines on feasibility studies by Thabane and colleagues (2016), formal hypothesis testing was not performed and therefore p values are not included in the analyses of these data. Microsoft Excel (2013) and SPSS (Version 22) (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses.

The complexity of the 2x2 repeated measures ANOVA with two within-factors makes sample size estimation for this design challenging (Potvin and Schutz, 2000). Furthermore, the effects of exercise on postprandial responses to OFTT have not been reported in OT2D. Therefore, the sample size was estimated in a healthy non-OT2D group, required to detect differences between the main effects for the carbohydrate conditions and the exercise conditions using a one-way repeated measures ANOVA design with two measures for each condition. Based on previous data in a healthy non-OT2D group (Weiss et al., 2008) it was estimated that the repeatability of the primary outcome TG AUC would be high (ICC=0.83). However, there are no available data to estimate the repeatability of an OT2D group. As such, a more conservative estimate of  $\rho=0.7$ , an effect size of 0.8, an alpha value of 0.05 and 80% power, was used to estimate that a sample size of 8 participants would be required to determine an effect in a healthy population. Therefore a sample size of 8 was used for this feasibility study in OT2D.



### 4.3 Results

Eight (median age, 40 [33, 48] years; BMI,  $28.3 \pm 4.6 \text{ kg}\cdot\text{m}^{-2}$ ) of nine adult male OT2D completed all study visits. One participant was excluded because of high blood glucose during OGTT ( $>11.1 \text{ mmol}\cdot\text{l}^{-1}$  2 hours after glucose ingestion). The recruitment process is shown in *Figure 4.1*. Participant demographics of the 8 participants that completed the study are reported in *Table 4.1*. Four participants had a father with T2D, three participants had a mother with T2D and one participant both parents with T2D. Study recruitment and completion was achieved 11 months after start date (March 2015) of the study, meeting the feasibility criteria for a future adequately powered study to be achieved in a multi-centre trial. There were no adverse events during or after exercise or following OFTT. The OFTT was well tolerated by all participants. All participants completed the two prescribed exercise sessions (mean work rate:  $69 \pm 17 \text{ W}$ , energy expenditure for sessions 1 and 2:  $276 \pm 63$  and  $274 \pm 53 \text{ kcal}$ , heart rate for sessions 1 and 2:  $113 \pm 20 \text{ bpm}$ ,  $112 \pm 20 \text{ bpm}$ ).



**Figure 4.1** Flow diagram of study recruitment based on the template recommended in the CONSORT statement extension to randomised pilot and feasibility trials (Eldridge et al., 2016).

**Table 4.1** Participant demographics (mean (SD))

<b>Participant demographics</b>	
Age (years)*	40 (33, 48)
Weight (kg)	86.1 (9.1)
BMI (kg.m <sup>-2</sup> )	28.3 (4.6)
Waist circumference (m)	1.00 (0.09)
Waist:hip circumference ratio	0.95 (0.04)
Total cholesterol (mmol.l <sup>-1</sup> )**	5.4 (0.7)
Triglycerides (mmol.l <sup>-1</sup> )**	1.2 (0.5)
HDL cholesterol (mmol.l <sup>-1</sup> )**	1.3 (0.4)
LDL cholesterol (mmol.l <sup>-1</sup> )**	3.6 (0.8)
Glucose (mmol.l <sup>-1</sup> )**	5.5 (0.7)
Insulin (uIU.ml <sup>-1</sup> )	8.7 (6.6)
HOMA-IR**	2.1 (1.4)
Matsuda index	6.7 (4.4)
$\dot{V}O_2$ peak (ml.kg <sup>-1</sup> .min <sup>-1</sup> )	28.6 (7.4)
$\dot{V}O_2$ AT (ml.kg <sup>-1</sup> .min <sup>-1</sup> )	14.6 (3.3)

\*denotes median (quartile 1, quartile 3)

\*\*denotes blood samples taken in the fasted state

#### 4.3.1 Serum Triglyceride Responses

Mean (SEM) TG responses at each time point for each condition are presented in *Figure*

4.2. TG increased from baseline in all conditions and peaked at 3-4 hours.

##### 4.3.1.1 Total AUC

#### Two way repeated measures ANOVA

##### Rest vs Exercise

There was a small effect for lower TG AUC after the exercise conditions compared to the rest conditions (mean difference= -0.27 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI= -1.34 to 0.81 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta^2 = 0.05$ ).

##### HFLC vs HFHC

There was a large effect for lower TG AUC after the HFHC conditions compared to the HFHC conditions (mean difference= -0.55 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI= -1.72 to 0.62 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta^2 = 0.15$ ).

## Interaction

There was a large interaction effect for carbohydrate and exercise ( $\eta p^2 = 0.24$ ).

## Mean Differences (pairwise comparisons)

### HFLC OFTT

Exercise had a trivial effect for increased TG AUC after the HFLC OFTT compared to rest; there were no differences between the grand means for TG AUC between rest and exercise (mean difference = 0.17 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI = -1.03 to 1.37 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta p^2 = 0.02$ ).

### HFHC OFTT

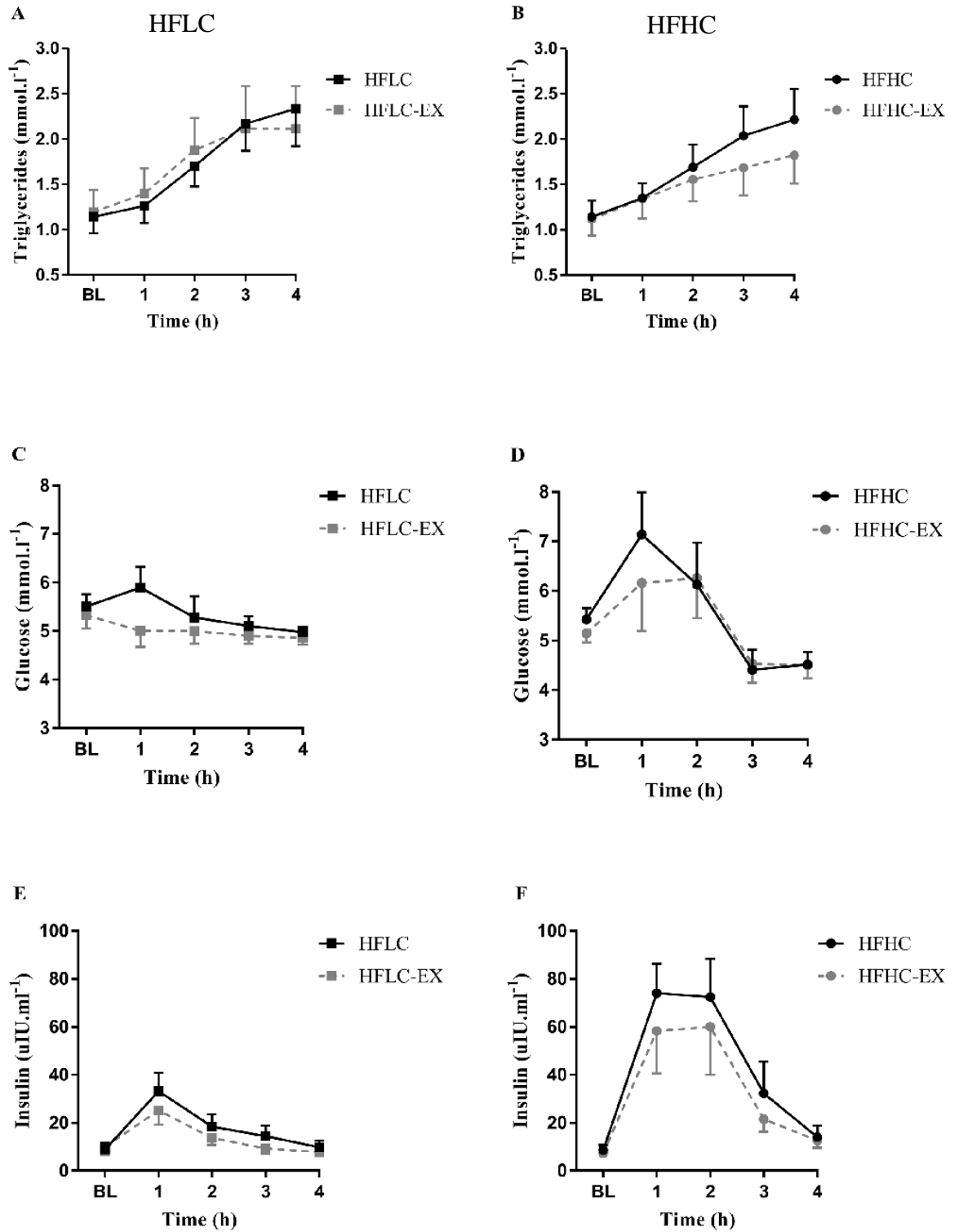
Exercise had a large effect for reducing TG AUC after the HFHC OFTT compared to rest; the confidence intervals are unequally distributed around zero and there was a large effect size in favour of a lower TG AUC for the exercise condition (mean difference = -0.71 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI = -2.06 to 0.64 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta p^2 = 0.18$ ).

### Rest

HFHC OFTT had a trivial effect for reducing TG AUC after rest compared to HFLC OFTT; there were no differences between the grand means for TG AUC between HFLC and HFHC OFTTs (mean difference = 0.11 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI = -0.97 to 1.19 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta p^2 = 0.01$ ).

### Exercise

HFHC OFTT had a large effect for reducing TAG AUC after exercise compared to HFLC OFTT; the confidence intervals are unevenly distributed around zero and there was an large effect size in favour of lower TG AUC for the HFHC OFTT (mean difference = -0.99 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI = -2.57 to 0.60 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta p^2 = 0.24$ ).



**Figure 4.2** Mean (standard error of the mean [SEM]) TG (panels A, B), glucose (panels C, D) and insulin (panels E, F) responses for; HFLC rest and exercise (EX) conditions (panels A, C & E), HFHC rest and exercise (EX) conditions (panels B, D & F).

#### 4.3.1.2 Incremental AUC

##### Two way repeated measures ANOVA

###### Rest vs Exercise

There was a large effect for lower TG iAUC in the exercise conditions compared to the rest conditions (mean difference=  $-0.30 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{l}^{-1}$ , CI=  $-0.76$  to  $0.17 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{l}^{-1}$ ,  $\eta^2= 0.25$ ).

###### HFLC vs HFHC

There was a large effect for lower TG iAUC after the HFHC conditions compared to the HFHC conditions (mean difference=  $-0.41 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{l}^{-1}$ , CI=  $-1.16$  to  $0.34 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{l}^{-1}$ ,  $\eta^2= 0.19$ ).

###### Interaction

There was a large interaction effect for carbohydrate and exercise ( $\eta^2= 0.32$ ).

##### Mean differences (pairwise comparisons)

###### HFHC OFTT

Exercise had no effect for increased TG iAUC after the HFHC OFTT compared to rest; there were no differences between the grand means for TG AUC between rest and exercise (mean difference=  $0.01 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{l}^{-1}$ , CI=  $-0.65$  to  $0.62 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{l}^{-1}$ ,  $\eta^2 < 0.001$ ).

###### HFHC OFTT

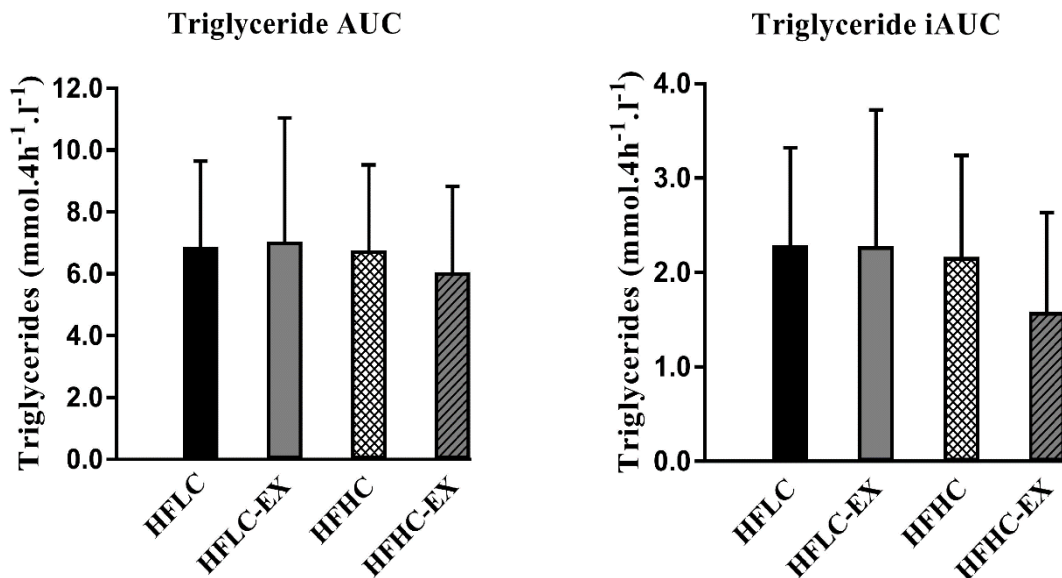
Exercise had a large effect for reducing TG iAUC after the HFHC OFTT compared to rest; the confidence intervals do not cross zero and there was a large effect size in favour of a lower TG iAUC for the exercise condition (mean difference=  $-0.58 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{l}^{-1}$ , CI=  $-1.15$  to  $-0.21 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{l}^{-1}$ ,  $\eta^2= 0.46$ ).

## Rest

HFHC OFTT had a small effect for reducing TG iAUC after rest compared to HFLC OFTT; there were no differences between the grand means for TG iAUC between the HFLC and HFHC OFTTs (mean difference=  $-0.13 \text{ mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$ , CI=  $-0.70$  to  $0.44 \text{ mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$ ,  $\eta^2= 0.04$ ).

## Exercise

HFHC OFTT had a large effect for reducing TAG iAUC after exercise compared to HFLC OFTT; the confidence intervals are unevenly distributed around zero and there was an large effect size in favour of lower TG iAUC for the HFHC OFTT (mean difference=  $-0.70 \text{ mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$ , CI=  $-1.74$  to  $0.35 \text{ mmol}\cdot 4\text{h}^{-1}\cdot \text{l}^{-1}$ ,  $\eta^2= 0.26$ ).



**Figure 4.3** Mean (SD) TG AUC (left) and iAUC (right) for each study condition

### 4.3.1.3 Baseline TG

There were no differences in TG at baseline between the rest and exercise conditions (mean difference=  $0.01 \text{ mmol}\cdot \text{l}^{-1}$ , CI=  $-0.16$  to  $0.19 \text{ mmol}\cdot \text{l}^{-1}$ ,  $\eta^2= 0.004$ ). There was a small effect for lower TG at baseline for the HFHC conditions compared to the HFLC conditions (mean difference=  $-0.04 \text{ mmol}\cdot \text{l}^{-1}$ , CI=  $-0.22$  to  $0.14 \text{ mmol}\cdot \text{l}^{-1}$ ,  $\eta^2= 0.04$ ).

There was a moderate interaction effect between carbohydrate content and exercise ( $\eta p^2 = 0.07$ ).

**Table 4.2** Mean (SD) postprandial responses to OFTT

	<b>HFLC</b>	<b>HFLC-EX</b>	<b>HFHC</b>	<b>HFHC-EX</b>
<b>TG AUC (mmol·4h<sup>-1</sup>·l<sup>-1</sup>)</b>	6.9 (2.8)	7.0 (4.0)	6.8 (2.8)	6.1 (2.8)
<b>TG iAUC (mmol·4h<sup>-1</sup>·l<sup>-1</sup>)</b>	2.3 (1.0)	2.3 (1.4)	2.2 (1.1)	1.6 (1.1)
<b>TG baseline (mmol·l<sup>-1</sup>)</b>	1.1 (0.5)	1.2 (0.7)	1.1 (0.5)	1.1 (0.5)
<b>Glucose AUC (mmol·4h<sup>-1</sup>·l<sup>-1</sup>)</b>	21.5 (3.2)	20.0 (2.4)	22.7 (5.7)	21.8 (5.3)
<b>Insulin AUC (uIU·4h<sup>-1</sup>·ml<sup>-1</sup>)</b>	75.5 (51.1)	57.0 (34.8)	190.2 (107.5)	149.8 (110.7)
<b>Cholesterol AUC (mmol·4h<sup>-1</sup>·l<sup>-1</sup>)</b>	21.2 (2.6)	21.5 (3.2)	21.4 (3.9)	21.3 (2.6)
<b>HDL-c AUC (mmol·4h<sup>-1</sup>·l<sup>-1</sup>)</b>	5.0 (1.6)	4.8 (1.6)	4.9 (1.4)	4.9 (1.5)

#### 4.3.2 Post-hoc sample size calculation for TG AUC and TG iAUC

Using the interaction data (mean difference and SD) for the reduction in TG AUC and TG iAUC with the HFHC OFTT and exercise, for 80% power and an alpha level of 0.05, a sample size of 28 participants would be required to detect a difference between TG AUC and 20 participants would be required to detect a difference between TG iAUC.

#### 4.3.3 Glucose AUC

##### Rest vs exercise

There was a large effect for lower blood glucose AUC after the exercise conditions compared to the rest conditions (mean difference = -1.20 mmol·4h<sup>-1</sup>·l<sup>-1</sup>, CI = -2.70 to 0.30 mmol·4h<sup>-1</sup>·l<sup>-1</sup>,  $\eta p^2 = 0.34$ ).



#### HFLC vs HFHC

There was a large effect for higher blood glucose after the HFHC conditions compared to the HFLC conditions (mean difference= 1.45 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI= -1.32 to 4.22 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta^2= 0.18$ ).

#### Interaction

There was a moderate interaction effect between carbohydrate and exercise conditions ( $\eta^2=0.08$ ).

### 4.3.4 Insulin AUC

#### Rest vs exercise

There was a large effect for lower insulin AUC after the exercise conditions compared to the rest conditions (mean difference= -29.4 uIU.4hour<sup>-1</sup>.ml<sup>-1</sup>, CI= -64.5 to 5.7 uIU.4hour<sup>-1</sup>.ml<sup>-1</sup>,  $\eta^2= 0.36$ ).

#### HFLC vs HFHC

Insulin AUC was higher in the HFHC compared to the HFLC conditions (mean difference= 103.8 uIU.4hour<sup>-1</sup>.ml<sup>-1</sup>, CI= 37.2 to 170.2 uIU.4hour<sup>-1</sup>.ml<sup>-1</sup>,  $\eta^2=0.66$ ).

#### Interaction

There was a moderate interaction effect between carbohydrate content and exercise conditions ( $\eta^2=0.06$ ).

### 4.3.5 Total cholesterol AUC

#### Rest vs exercise

There were no differences between total cholesterol AUC for rest and exercise conditions (mean difference= -0.63 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI= -1.01 to 0.89 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta^2= 0.003$ ).

#### HFHC vs HFLC

There were no differences between cholesterol AUC for HFHC and HFLC conditions (mean difference= -0.01 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI= -0.91 to 0.90 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta^2 < 0.001$ ).

#### Interaction

There was no interaction effect between carbohydrate and exercise conditions ( $\eta^2 = 0.02$ ).

### 4.3.6 HDL-c AUC

#### Rest vs exercise

There was a large effect for lower HDL-c AUC after the exercise conditions compared to the rest conditions (mean difference= -0.13 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI= -0.35 to 0.09 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta^2 = 0.22$ ).

#### HFHC vs HFLC

There were no differences in HDL-c AUC between the HFHC and HFLC conditions (mean difference= -0.03 mmol.4h<sup>-1</sup>.l<sup>-1</sup>, CI= -0.22 to 0.15 mmol.4h<sup>-1</sup>.l<sup>-1</sup>,  $\eta^2 = 0.03$ ).

#### Interaction

There was a large effect for an interaction effect between carbohydrate and exercise conditions for HDL-c AUC ( $\eta^2 = 0.15$ ).

## 4.4 Discussion

This feasibility study investigated postprandial TG responses of adult male OT2D to HFHC and HFHC OFTTs, with both meals preceded by rest and exercise. Recruitment rate was slow but fell within the a priori limits to determine that it is feasible for a future adequately powered multi-centre study to be conducted. Adherence following recruitment was good, and the OFTTs and exercise interventions were well tolerated by

the participants. TG AUC and TG iAUC showed large interaction effects for lower TG AUC when exercise was combined with the HFHC OFTT. Although these findings should be interpreted with caution, it is interesting that the lowest TG response was observed in the HFHC condition combined with exercise. In agreement with mechanistic findings from Rabøl and colleagues (2011), these findings may suggest that acute improvement in muscle insulin sensitivity is a key mechanism involved in reducing postprandial TG after mixed meal fat ingestion. However, a larger, adequately powered study is required to confirm these findings.

#### 4.4.1 Are exercise induced increases in insulin sensitivity and reduced postprandial TG related?

The effectiveness of the intervention was evaluated in order to calculate the sample size required for an adequately powered study. Our feasibility data indicate that 1-hour of acute moderate intensity exercise (90% AT) prior to the HFHC conditions showed large effect sizes for lower TG responses (AUC and iAUC) compared to the rest condition. In contrast, the HFLC condition did not reduce postprandial TG compared to the rest condition. The apparent tendency for lower TG concentrations after acute exercise within HFHC conditions could be related to acute changes in insulin sensitivity and therefore requires further evaluation. The more frequently cited mechanisms of reduced TG response after exercise include, upregulation of LPL and VLDL1 modification for increased postprandial TG removal (Ghafouri et al., 2015, Kersten, 2014). However, exercise induced upregulation of LPL was not observed despite reductions in postprandial TG compared to the non-exercise OFTT (Harrison et al., 2012) and VLDL1 modification explains up to 50% of the reduction in postprandial TG (Al-Shayji et al., 2012). Therefore, there are likely to be other underlying mechanisms that reduce

postprandial TG. Whilst these data must be interpreted with caution, data from this feasibility study may indicate that improved insulin sensitivity may contribute to the reduction in postprandial TG, this is supported by a mechanistic study (Rabol et al., 2011), described below.

It has been cited that exercise-induced improvements in insulin sensitivity do not influence reductions in postprandial TG (Gill et al., 2002, Maraki and Sidossis, 2013). Gill and colleagues (2002) evaluated postprandial TG responses to a HFHC OFFT with and without prior exercise in healthy groups. They reported that because the observed reductions in postprandial TG and insulin concentrations after exercise compared to rest were not correlated, acute improvements in insulin sensitivity and TG reduction are not related (Gill et al., 2002). However, there are several limitations with this study that should be considered:

1. Correlations do not demonstrate cause and effect.
2. This study employed only HFHC (approximately 1.2 g/kg body mass of fat and 1.2 g/kg body mass of carbohydrate) OFTTs and therefore have only assessed postprandial TG responses under hyperinsulinaemic conditions in apparently healthy participants. OFTTs containing high carbohydrate reduces postprandial TG in healthy individuals (without exercise intervention) compared to HFLC OFTTs (Kriketos et al., 2005, Kriketos et al., 2003) in a dose related pattern (Cohen and Berger, 1990). High carbohydrate may therefore confound the capacity to assess the effects of exercise-induced reductions in postprandial TG concentrations in healthy individuals.
3. There is likely a ceiling effect for improvements in insulin sensitivity (Magkos et al., 2008a); only assessing responses of insulin sensitive participants may not allow this relationship to be evaluated.

4. Hepatic production of TG, TG clearance and insulin sensitivity were not directly measured which limits the strength of the conclusions.

An arguably more robust method to assess mechanisms underlying postprandial responses involves complex metabolic studies using stable isotopes and MRS, such as those conducted by Petersen and colleagues (2007) and Rabøl and colleagues (2011). Using  $^{13}\text{C}$  and  $^1\text{H}$  MRS and deuterium ( $2\text{H}_2\text{O}$ ) ingestion and measurements to evaluate the effects of prior (8 hours before 2<sup>nd</sup> meal was consumed) acute exercise (3x 15 minutes at 75-85% calculated maximum heart rate) on postprandial muscle and liver glycogen and TG metabolism, Rabøl and colleagues (2011) showed a 3 fold increase in muscle glycogen synthesis, a 40% decrease in hepatic TG production and a 27% reduction in de novo lipogenesis. These data indicate the relatedness of insulin sensitivity and carbohydrate and lipid metabolism and highlight the importance of insulin sensitivity in postprandial TG metabolism.

The assessment of TG responses to low and high circulating insulin concentrations in the present study provides an alternative assessment of the role of insulin responses and inferred changes in insulin sensitivity with exercise. The large effect sizes for lower glucose AUC and insulin AUC after the exercise conditions are in keeping with previous studies that have evaluated postprandial glucose and insulin concentrations after OFTT. These findings are considered to demonstrate improved insulin sensitivity with exercise interventions (Gill et al., 2002). However, the capacity to evaluate direct mechanisms is limited compared to Rabøl and colleagues (2011).

#### 4.4.2 Does OFTT carbohydrate content influence postprandial TG in OT2D?

Another objective of the full adequately powered study would be to determine whether high carbohydrate content reduced postprandial TG in OT2D. In agreement with the findings of Kriketos and colleagues (Kriketos et al., 2005), the pairwise comparisons revealed trivial mean differences between postprandial TG in the HFHC and HFLC rest conditions. This may be explained by inability of insulin to suppress hepatic production of VLDL as proposed by Kriketos and colleagues (Kriketos et al., 2005), consistent with work by Johanson and colleagues (Johanson et al., 2004). Unlike Kriketos and colleagues (Kriketos et al., 2005), the present study did not include a 'healthy' control group to support this finding being a deviation from normal responses. As discussed, in apparently healthy people (excluding OT2D), the postprandial TG response to high fat intake is reduced when carbohydrate content is increased (Kriketos et al., 2003, Kriketos et al., 2005, Cohen and Berger, 1990). The mechanisms contributing to this are thought to be; reduced hepatic VLDL production mediated by hyperinsulinaemia, and delayed gastric emptying due to raised carbohydrate and elevated circulating insulin (Cohen and Berger, 1990). In the present study, there was not a reduced TG AUC after HFHC compared to HFLC in the rest conditions. If hepatic production of VLDL was reduced it was not sufficient to reduce total TG responses. Another explanation is that DNL increases in OT2D as a result of increased carbohydrate delivery to the liver (Petersen et al., 2007). This may offset the influence of insulin on circulating TG, however, a further mechanistic study would be required to determine this. The TG responses after HFHC and HFLC meals in the rest conditions are different to TG responses observed in studies that demonstrated delayed gastric emptying with HFHC (Cohen and Berger, 1990). It is therefore unlikely that delayed gastric emptying influenced the postprandial responses.

#### 4.4.3 Strengths and Limitations

The strengths of this feasibility study include the robust design which enabled insight into the role of acute exercise, carbohydrate content of OFTTs and insulin concentrations on postprandial TG responses in OT2D. The present findings raise some pertinent issues within this area of investigation. However, these must be interpreted with caution as this study was not adequately powered to detect statistical differences between data. A limitation of this study is the inability to assess the specific underlying mechanisms of the apparent interaction between prior acute exercise and the HFHC OFTT which led to a “large effect” for a reduction in both TG AUC and iAUC responses. The use of isotope tracers within the test meals, in a similar manner to those used by Rabøl and colleagues (2011), could be considered to evaluate underlying mechanisms if this pattern within the data remained in a larger, adequately powered study.

#### 4.5 Conclusions

The present study design is feasible for a future multi-centre trial. Exercise had no effect on reducing postprandial TG after HFLC and may reduce postprandial TG after HFHC OFTTs in OT2D. In support of previous findings, carbohydrate content did not alter postprandial TG responses when participants rested the day before the OFTT. Future work should investigate the effects of exercise, carbohydrate content and insulin sensitivity on postprandial TG responses in male and female sedentary adults with reduced insulin sensitivity.

## Chapter 5: The effects of acute interval exercise and strawberry intake on postprandial lipaemia

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[http://journals.lww.com/acsm-msse/Abstract/publishahead/The\\_Effects\\_of\\_Acute\\_Interval\\_Exercise\\_and.97184.aspx](http://journals.lww.com/acsm-msse/Abstract/publishahead/The_Effects_of_Acute_Interval_Exercise_and.97184.aspx)

### 5.1 Introduction

Impaired lipid handling after oral fat ingestion results in increased circulating lipids and associated metabolic stress for prolonged time periods. This postprandial characteristic is often reported in physical inactivity, obesity and type 2 diabetes and is strongly associated with atherosclerosis (Pirillo et al., 2014). Acute endothelial dysfunction, increased inflammation and oxidative stress occur during postprandial lipaemia and may contribute to an atherogenic environment (de Vries et al., 2014, Tsai et al., 2004). Furthermore, elevated circulating postprandial lipids likely increase the propensity for oxidation of lipids, such as LDL, which are key protagonists of atherosclerosis (Graner et al., 2006). Attenuation of the postprandial TG response, total and oxidised LDL (oxLDL), is therefore likely to be beneficial for optimising long-term cardiovascular and metabolic health, particularly in overweight or obese individuals.

Exercise performed acutely before a high fat meal (typically 4-24 hours prior to meal ingestion) reduces postprandial TG (for a recent review see; (Freese et al., 2014)). Many studies have investigated the effects of continuous moderate intensity exercise, with most showing favourable postprandial responses after exercise. These studies have been



reviewed in detail elsewhere (Freese et al., 2014). Interval exercise involving several bursts of high intensity exercise (lasting 6 to 240 s) interspersed with light exercise is also an effective strategy to reduce postprandial lipaemia but few studies have been conducted (for a recent review see; (Burns et al., 2015)). When compared to moderate intensity continuous exercise, submaximal high intensity interval exercise has been shown to be similar (Ferreira et al., 2011), or more effective (Trombold et al., 2013), at reducing postprandial TG. Supramaximal high intensity exercise has the added benefit of reducing the time required to complete a fixed amount of work compared to exercise of lower intensities (Little et al., 2010). Although this is appealing, because lack of time to exercise is a common reason for people not performing exercise (Burns et al., 2015, Little et al., 2010), the practicality (Little et al., 2010) and safety (Eskelinen et al., 2016b) of supramaximal exercise is not fully understood in sedentary populations. As such, the use of submaximal high intensity interval exercise to lower PPL may be warranted. However, few studies have investigated this mode of exercise on modifying postprandial lipaemia within adults at higher metabolic risk (Burns et al., 2015).

Having a healthy diet is inversely related to CVD and all-cause mortality (Wang et al., 2014). Consuming sufficient portions of fruit and vegetables each day is an important component of a healthy diet, according to international guidelines (Joint WHO/FAO Expert Consultation, 2003). In addition to being rich in dietary fibre and essential nutrients, many fruits and vegetables are functional foods; those that provide health benefits in addition to basic nutrition (Basu et al., 2014b). The strawberry is considered to be a functional food due to its antioxidant, anti-inflammatory, antihypertensive and lipid lowering effects (for a recent review see; (Basu et al., 2014b)). The high content of phenols (which include; anthocyanins, catechins, ellagitannins, perlargonidins and quercetin) within strawberries are proposed to be important for modifying circulating lipids and lipid oxidation in the postprandial period (Burton-Freeman et al., 2010).

Consumption of 10g freeze dried strawberries (equivalent to 110g fresh weight strawberries) with a moderate fat (31g) high carbohydrate (135g) meal compared to a placebo acutely reduced postprandial TG, oxLDL, and markers of inflammation (C-reactive protein, Interleukin-6) in overweight men and women (Burton-Freeman et al., 2010, Edirisinghe et al., 2011). However, the acute effects of strawberries on the postprandial responses to a high-fat, low-carbohydrate meal has, to the author's knowledge, not been investigated. This is important to help fully understand the potential use of strawberry intake in reducing postprandial cardio-metabolic stresses associated with fat ingestion.

Prior submaximal high intensity interval exercise and strawberry consumption appear to be independently beneficial in acutely reducing lipid-induced metabolic dysregulation after moderate or high fat meal ingestion. However, the combined effect of these lifestyle interventions has not been investigated to date. The aim of this study was to investigate the separate and combined effects of prior acute exercise and strawberry consumption on reducing postprandial TG responses and oxidative stress after an oral fat tolerance test (OFTT) in inactive overweight and obese adult males. It was hypothesised that exercise and strawberry interventions would independently reduce postprandial TG and that an interaction effect for strawberry and exercise in reducing postprandial TG would be observed.

## 5.2 Methods

### 5.2.1 Participants

Overweight and obese adult males (BMI > 25 kg.m<sup>-2</sup>, waist circumference > 94 cm) with no known cardio-metabolic disorders were recruited. Participants were excluded if they

met the exclusion criteria listed in the General methods (Chapter 2). This study was conducted according to the declaration of Helsinki and approved by the Department of Sport, Health and Exercise Science Ethics Committee, University of Hull. Written informed consent was given by all participants before study commencement.

### 5.2.2 Study design

This randomised, single blinded, crossover study investigated the separate and combined effects of acute prior exercise and acute strawberry consumption on postprandial lipaemic responses (serum TG concentrations) and oxidative stress responses (serum oxLDL and lipid hydroperoxides). There were four experimental conditions which included either an abbreviated OFTT meal containing strawberry milkshake mix [(placebo), 20g, Tesco, UK] or freeze dried strawberries [(intervention), 25g, European Freeze Dry Ltd, Preston, UK] (detailed in Chapter 2). The OFTT meals were preceded by either rest or submaximal high intensity interval exercise (detailed in Chapter 2) conducted on the day before OFTT. Each participant completed all experimental conditions, these were; 1. Placebo OFTT rest condition (R-P), 2. Strawberry OFTT rest condition (R-S), 3. Placebo OFTT exercise condition (Ex-P), 4. Strawberry OFTT exercise condition (Ex-S). Participants attended the research laboratory before 10:00am on four separate occasions, separated by at least 72 hours. During the acute exercise conditions, participants attended the laboratory after 3:30pm, 16 to 18 hours before the scheduled OFTT. The order in which the trial conditions were performed was randomised a priori for each participant. All tests were completed within 8 weeks of the screening visit.

### 5.2.3 Screening visit

Participants fasted for 2 hours before the screening visit. Baseline stature (Harpenden Stadiometer, Holtain Limited, Crymych Pembrokeshire), body mass (Seca Balance Scales, Seca, Hamburg, Germany), waist and hip circumferences (Seca 201 ergonomic circumference measuring tape, Hamburg, Germany) were measured and body fat content (percentage) was estimated using bioimpedance (BF900 Maltron Body Composition Analyser, Essex, UK). Blood pressure (Omron M6, Omron Healthcare LTD, Milton Keynes, UK) and resting ECG measurements (GE CASE system, GE Healthcare, Freiburg, Germany) were taken and this was followed by a symptom-limited maximal CPET to volitional exhaustion (detailed in Chapter 2).

### 5.2.4 Visits 1-4

Participants randomised to the exercise condition attended the laboratory the afternoon before the OFTT having refrained from exercise that day. Participants randomised to the rest condition refrained from exercise 24 hours before OFTT and did not attend the laboratory. All participants were provided with a commercial “ready meal” (detailed in Chapter 2) to consume as their only nutritional intake on every evening preceding an OFTT study visit. Participants attended the laboratory before 10am the following morning having fasted overnight (>10 hours). After 10 minutes of rest, three blood pressure measurements were taken over a period of 10 minutes. A cannula was inserted into a vein in the antecubital fossa and a blood sample was drawn. Once the participant was provided with an OFTT meal, they were invited to consume it within 5 minutes. The OFTT meal either contained freeze dried strawberries (intervention) or strawberry flavouring (placebo). A blood sample was drawn on the hour for 4 hours after OFTT meal ingestion.

### 5.2.5 Biochemistry analysis

Serum TG, total cholesterol, high density lipoprotein cholesterol (HDL-c), and plasma glucose were measured at every time point. Low Density Lipoprotein (LDL-c) was estimated from the Friedewald equation (Friedewald et al., 1972) at every time point. Serum oxLDL was determined by using an ELISA (Merckodia Inc, Uppsala, Sweden), and serum lipid peroxidation was estimated by using the FOX1 assay. These measures were taken at baseline and 4 hours and were measured in duplicate. The Folin-Ciocalteu assay was used to estimate the anti-oxidant capacity of the strawberry and the placebo product.

### 5.2.6 Outcome measures

The primary outcome was TG AUC during OFTT. Secondary outcome measures were TG iAUC, oxLDL and lipid peroxidation (FOX1 assay).

### 5.2.7 Statistical Analyses

Normal (Gaussian) distribution of data was verified using the Shapiro-Wilk test, tests for skewness and kurtosis of distributions and visual inspection of histogram charts was conducted. Non-normally distributed data were analysed using non-parametric analyses. Data are presented as mean and standard deviation (SD) for normal data, and non-normally distributed data are presented as median and quartiles 1 and 3 (Q1, Q3). Total area under the curve (AUC) and incremental AUC (iAUC) for TG, cholesterol, HDL-c and glucose was determined by the trapezoidal method (Matthews et al., 1990). oxLDL and lipid hydroperoxides were measured at baseline and at 4 hours and the difference between baseline and 4 hours was calculated. To assess the differences between

outcome measures for each trial condition, 2x2 repeated measures analysis of variance (ANOVA) was used. Specifically, activity (exercise/ no exercise) was treated as a study condition and nutritional content (strawberry/ no strawberry) was treated as a study condition. Each activity/nutritional intervention and placebo appeared twice across the study trials therefore the 2x2 repeated measures ANOVA enabled the influence of exercise and strawberry to be assessed independently across the study and the interaction revealed whether a combination of the study conditions influenced postprandial responses. Mean difference with 95% confidence intervals (CI), p values and effect sizes using partial eta squared ( $\eta^2$ ) are reported. The alpha level was set at 0.05, and  $\eta^2$  was used to determine the effect size with small, medium and large effects set at 0.01, 0.06 and 0.14, respectively (Cohen, 1988). Where significance was reached, post hoc pairwise comparisons were made with Bonferroni adjustment and reported as mean difference, CI, p values and  $\eta^2$ . Microsoft Excel (2013) and SPSS (Version 22) (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses.

The complexity of the 2x2 repeated measures ANOVA with two within factors makes sample size estimation for this design challenging (Potvin and Schutz, 2000). As such, a sample size required to detect differences between the main effects for the diet condition and the exercise condition using a one way repeated measures ANOVA design with two measures for each condition was estimated. Based on previous data (Weiss et al., 2008) it was expected that the repeatability of the primary outcome TG AUC would be high (ICC=0.83). Using a more conservative estimate of  $\rho=0.7$ , an effect size of 0.7, an alpha value of 0.05 and 80% power a sample size of 10 participants was obtained.

### 5.3 Results

Ten of eleven males (median age, 31.5 Q1, 28.5 Q3, 46.3 years; mean  $\pm$ SD BMI, 29.9  $\pm$ 1.8 kg·m<sup>-2</sup>; waist circumference: 1.05  $\pm$ 0.05 m) completed all study visits. Demographics for these participants are reported in *Table 5.1*. One participant dropped out of the study after the screening visit for personal reasons. Six participants were overweight (BMI 25 kg·m<sup>-2</sup> to 30 kg·m<sup>-2</sup>), four were obese (BMI >30 kg·m<sup>-2</sup>) and all were inactive (defined by self-reported exercise <150 minutes per week). All participants completed the two submaximal high intensity interval exercise protocols which lasted one hour in total. The peak heart rates achieved during exercise were 93  $\pm$ 4% of peak heart rates measured in CPET and there were no differences in peak heart rates between the two interventions (p= 0.504). The mean (SD) work rate (W) for the low and high intensity intervals were 48  $\pm$ 16 W and 181  $\pm$ 49 W, respectively. The Folin-Ciocalteu assay identified that freeze dried strawberry had 4.5 fold greater phenolic capacity compared to the placebo (895 mg vs. 194 mg). There were no adverse effects during or following the exercise interventions or high fat meal ingestion.

**Table 5.1** Mean (SD) Baseline Demographics

<b>Baseline measures</b>	
<b>Age (years)*</b>	31.5 (28.5, 46.3)
<b>Body mass (kg)</b>	91.9 (6.8)
<b>BMI (kgm<sup>-2</sup>)</b>	29.9 (1.8)
<b>Waist circumference (m)</b>	1.05 (0.05)
<b>Waist to hip ratio</b>	0.97 (0.05)
<b>Body fat (%)</b>	25.5 (5.2)
<b>Blood Pressure (mmHg)</b>	129 (7)/ 80 (9)
<b><math>\dot{V}O_2</math> peak (ml·kg<sup>-1</sup>·min<sup>-1</sup>)*</b>	33.2 (26.7, 36.5)
<b><math>\dot{V}O_2</math> AT (ml·kg<sup>-1</sup>·min<sup>-1</sup>)*</b>	18.1 (15.8, 19.3)
<b>Triglycerides (mmol·l<sup>-1</sup>)</b>	1.3 (0.4)
<b>HDL (mmol·l<sup>-1</sup>)</b>	1.2 (0.1)
<b>Cholesterol (mmol·l<sup>-1</sup>)</b>	5.3 (1.1)
<b>Glucose (mmol·l<sup>-1</sup>)*</b>	5.2 (4.9, 5.4)

\*Reported as median (IQ1, IQ3)

### 5.3.1 Serum TG responses to OFTT

Mean (SD) TG responses at each time point for each condition are presented in *Figure 5.1*. TG increased from baseline in all conditions and peaked at 3-4 hours.

#### 5.3.1.1 Total AUC

TG AUC was 1.5 mmol·4h<sup>-1</sup>·L<sup>-1</sup> lower (95% confidence interval [CI] = -2.3 to -0.8, p= 0.001,  $\eta^2= 0.71$ ) for the two exercise conditions compared to the two resting conditions. Post hoc pairwise comparisons with Bonferroni adjustment identified that TG AUC was 1.6 mmol·4h<sup>-1</sup>·L<sup>-1</sup> lower in the exercise condition compared to rest condition for the placebo OFTT (CI= -2.5 to -0.5, p= 0.009,  $\eta^2= 0.55$ ) and by 1.5 mmol·4h<sup>-1</sup>·L<sup>-1</sup> for the strawberry OFTT (CI= -2.9 to -0.2, p= 0.033,  $\eta^2= 0.41$ ). There were no differences in TG AUC between the strawberry OFTT and placebo OFTT (Mean difference= -0.3 mmol·4h<sup>-1</sup>·L<sup>-1</sup> CI= -1.3 to 0.7, p= 0.475,  $\eta^2= 0.06$ ). There was no exercise and strawberry interaction (p= 0.970,  $\eta^2 < 0.001$ ).

#### 5.3.1.2 Incremental AUC

There was a large effect size for lower TG iAUC (Mean difference= 0.4 mmol·4h<sup>-1</sup>·L<sup>-1</sup>, CI = -0.2 to 1.1, p= 0.175,  $\eta^2= 0.19$ ) in the exercise conditions compared to the resting conditions. TG iAUC was 0.5 mmol·4h<sup>-1</sup>·L<sup>-1</sup> lower in the placebo conditions than the strawberry conditions (CI= -1.0 to -0.1, p= 0.021,  $\eta^2= 0.47$ ). Post hoc analyses identified that TG iAUC was 0.7 mmol·4h<sup>-1</sup>·L<sup>-1</sup> lower for the placebo condition compared to strawberry condition with exercise (CI= -1.1 to -0.3, p= 0.005,  $\eta^2= 0.61$ ) but not with rest (mean difference= 0.4 mmol·4h<sup>-1</sup>·L<sup>-1</sup>, CI= -1.2 to 0.5, p= 0.331,  $\eta^2= 0.11$ ). There was no interaction between conditions (p= 0.516,  $\eta^2= 0.05$ ).

#### 5.3.1.3 Baseline

Baseline TG was 0.3 mmol·4h<sup>-1</sup>·L<sup>-1</sup> lower (CI= -0.4 to 0.2, p=0.001,  $\eta^2= 0.74$ ) in the exercise conditions compared to the resting conditions. Post hoc analyses identified that

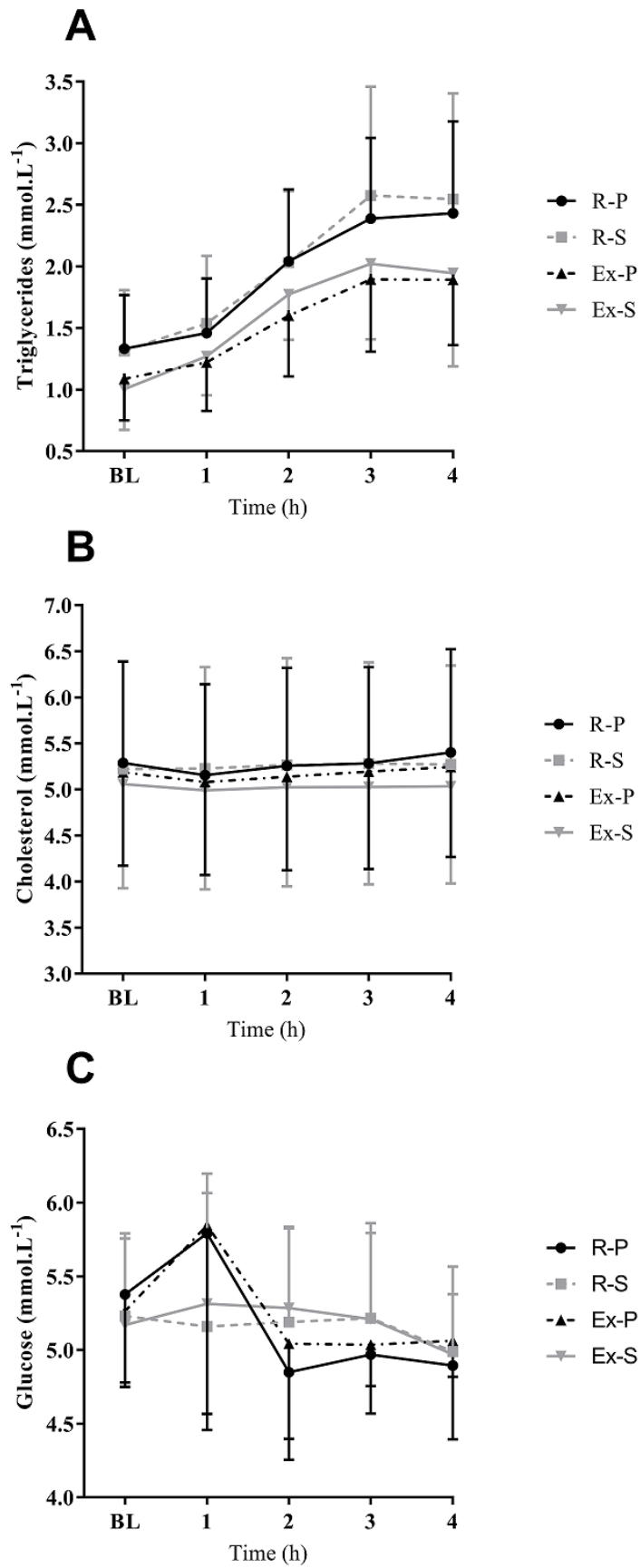


baseline TG was  $0.2 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$  lower with exercise compared to rest condition with the placebo (CI= -0.4 to -0.1,  $p=0.011$ ,  $\eta^2= 0.53$ ) and  $0.3 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$  lower with the strawberry condition (CI= -0.5 to -0.1,  $p=0.014$ ,  $\eta^2= 0.50$ ). There were no differences in baseline TG in the strawberry conditions compared to the placebo conditions (Mean difference=  $0.1 \text{ mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}$ , CI= -0.1 to 0.2,  $p=0.484$ ,  $\eta^2=0.06$ ). There was no interaction effect between conditions ( $p=0.660$ ,  $\eta^2=0.02$ ).

**Table 5.2** Mean (SD) postprandial responses for each study condition

	<b>Rest &amp; Placebo</b>	<b>Rest &amp; Strawberry</b>	<b>Exercise &amp; Placebo</b>	<b>Exercise &amp; Strawberry</b>
<b>TG AUC (<math>\text{mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}</math>)</b>	7.8 (2.2)	8.1 (2.6)	6.2 (1.8)*	6.5 (1.7)*
<b>TG iAUC (<math>\text{mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}</math>)</b>	2.4 (0.8)**	2.8 (1.2)	1.9 (0.8)**	2.5 (0.6)
<b>TG baseline (<math>\text{mmol}\cdot\text{L}^{-1}</math>)</b>	1.3 (0.4)	1.3 (0.5)	1.1 (0.3)*	1.0 (0.3)*
<b><math>\Delta</math> oxLDL (<math>\text{mU}\cdot\text{L}^{-1}</math>)</b>	-10.7 (15.7)	0.4 (14.2)	1.1 (9.4)	-4.0 (10.6)
<b><math>\Delta</math> Lipid hydroperoxides (<math>\mu\text{mol}\cdot\text{L}^{-1}</math>)</b>	11.6 (10.1)	8.0 (11.7)	4.4 (11.6)	13.6 (16.6)
<b>Cholesterol AUC (<math>\text{mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}</math>)</b>	21.0 (4.2)	21.0 (4.5)	20.6 (4.1)*	20.1 (4.3)*
<b>HDL AUC (<math>\text{mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}</math>)</b>	4.7 (0.6)	4.6 (0.4)	4.6 (0.5)	4.6 (0.4)
<b>Glucose AUC (<math>\text{mmol}\cdot\text{4h}^{-1}\cdot\text{L}^{-1}</math>)<sup>#</sup></b>	20.0 (4.3)	19.8 (1.8)	20.6 (2.5)	20.2 (1.5)

\*lower for the exercise conditions ( $p<0.05$ ) \*\*lower for the placebo conditions ( $p<0.05$ ) <sup>#</sup>Glucose is expressed as median (interquartile range)



**Figure 5.1** TG (Panel A), cholesterol (Panel B) and glucose (Panel C) responses to OFTT

### 5.3.2 Oxidative stress responses to OFTT

Mean (SD) change ( $\Delta$ ) in oxLDL and lipid hydroperoxides from baseline to 4 hours are reported in *Table 5.2*. There were no differences in oxLDL for the exercise (Mean difference=  $-3.6 \text{ mU}\cdot\text{L}^{-1}$ , CI=  $-14.3$  to  $7.0$ ,  $p= 0.45$ ,  $\eta^2= 0.06$ ) or strawberry (Mean difference=  $-2.9 \text{ mU}\cdot\text{L}^{-1}$ , CI=  $-9.6$  to  $3.7$ ,  $p= 0.34$ ,  $\eta^2= 0.10$ ) conditions. However, there was a large interaction effect size between conditions ( $p= 0.16$ ,  $\eta^2= 0.21$ ). There were no differences in lipid hydroperoxides for the exercise (Mean difference=  $0.8 \mu\text{mol}\cdot\text{L}^{-1}$ , CI=  $-8.0$  to  $9.6$ ,  $p= 0.84$ ,  $\eta^2= 0.01$ ) or strawberry (Mean difference=  $-2.8 \mu\text{mol}\cdot\text{L}^{-1}$ , CI=  $-11.1$  to  $5.6$ ,  $p= 0.47$ ,  $\eta^2= 0.06$ ) conditions. However, there was a large interaction effect size between the conditions ( $p= 0.13$ ,  $\eta^2= 0.24$ ).

### 5.3.3 Cholesterol, HDL, and glucose responses to OFTT

The cholesterol, HDL, and glucose AUC in response to OFTT are presented in *Table 5.2*. Cholesterol AUC was  $0.7 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$  lower in the exercise conditions compared to the rest conditions (CI=  $-1.1$  to  $-0.2$ ,  $p= 0.01$ ,  $\eta^2= 0.58$ ). There was no effect for exercise (Mean difference=  $0.01 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$ , CI=  $-0.13$  to  $0.14$ ,  $p=0.94$ ,  $\eta^2=0.001$ ) or strawberry (Mean difference=  $0.03 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$ , CI=  $-0.06$  to  $0.14$ ,  $p=0.43$ ,  $\eta^2= 0.07$ ) conditions on HDL responses to OFTT. There was no effect for exercise (Mean difference=  $0.29 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$ , CI=  $-1.04$  to  $0.43$ ,  $p=0.387$ ,  $\eta^2=0.08$ ) or strawberry (Mean difference =  $0.14 \text{ mmol}\cdot 4\text{h}^{-1}\cdot\text{L}^{-1}$ , CI=  $-0.55$  to  $0.83$ ,  $p= 0.655$ ,  $\eta^2= 0.02$ ) on glucose responses to OFTT.

## 5.4 Discussion

The separate and combined effects of acute submaximal high intensity interval exercise and strawberry consumption on postprandial responses to OFTTs among overweight and obese adult males were investigated. It was demonstrated that acute submaximal high intensity interval exercise was effective in reducing TG AUC after OFTTs. This significant effect of acute exercise in lowering postprandial TG was evident both with and without strawberry consumption. However, contrary to the study hypotheses, strawberry consumption with OFTT ingestion did not alter TG AUC concentrations and there was no interaction between strawberry consumption and submaximal high intensity interval exercise. The secondary findings indicate that there was a large effect size observed for acute submaximal high intensity interval exercise reducing TG iAUC. Whereas, TG iAUC was increased with strawberry consumption. There were no significant changes in lipid related oxidative stress responses between conditions.

### 5.4.1 Exercise and postprandial triglycerides

There was a reduction in TG AUC in response to the OFTT by approximately 20% in the submaximal high intensity interval exercise conditions compared to the control conditions. Acute prior exercise significantly lowered baseline TG and there was a large effect size for lower TG iAUC which contributed to the reduction in total AUC. Reductions in TG AUC of a similar magnitude have been reported in response to moderate continuous exercise (Freese et al., 2014) and high intensity interval exercise (Trombold et al., 2013, Ferreira et al., 2011). An individualised submaximal high intensity interval exercise protocol consistent with exercise intensity domains identified by analysis of expired ventilatory gasses measured during a CPET was selected (Ozyener et al., 2001). Other submaximal high intensity interval exercise interventions

that have successfully reduced postprandial lipaemia lasted approximately 40 minutes and were stopped when participants had expended 500 kcal (Ferreira et al., 2011) or 660 kcal (Trombold et al., 2013). An older, more overweight, and less active population with higher mean fasting TG concentrations compared to previous studies was recruited for this study. For practical reasons (this is, to avoid unrealistic length of exercise sessions) and real life application, the 40 minute duration of high intensity interval exercise was predefined (rather than a target energy expenditure) and the effects of individualised interventions at clearly defined exercise intensities were investigated. This is important because cardiorespiratory fitness is inversely related to cardio-metabolic health. Accordingly, participants with lower levels of cardiorespiratory fitness, exercising at the same relative intensity, will need to exercise for longer than a fitter individual to attain the same overall energy expenditure. Given the frequently cited barriers to exercise being time, it is unrealistic to expect an individual with poor cardiorespiratory fitness to exercise to attain a high total energy expenditure (>500 kcal) as this would typically require exercise sessions in excess of one hour. An exercise session duration of greater than one hour is in excess of recommended target guidelines for apparently healthy populations, which are seldom met (Tucker et al., 2011). Therefore, investigating the effects of acute exercise by predefined a fixed amount of time may be more ecologically valid. Furthermore, standard equations used for calculating energy expenditure from expired oxygen and carbon dioxide are inaccurate during interval exercise that involves exercise intensities above the anaerobic threshold. Therefore, the validity of high intensity interval exercise interventions that use predefined estimated energy expenditure targets based on expired oxygen and carbon dioxide calculations could be questioned. Finally, as considered later, total energy expenditure may not be the key mechanism involved in reducing postprandial TG with high intensity interval exercise (Burns et al., 2015).

Interval exercise has the advantage of enabling a greater volume of work/energy expenditure to be completed within a period of time (Little et al., 2010, Trombold et al., 2013), as well as varying the physiological challenge on the body when compared to continuous moderate intensity exercise. High intensity interval exercise has superior levels of enjoyment (Heinrich et al., 2014), lower perceived work (Kilpatrick et al., 2016) and increased likelihood of continuing regular exercise (Heinrich et al., 2014, Kilpatrick et al., 2016) in addition to the numerous cardio-metabolic benefits (Little et al., 2010, Gibala et al., 2012) compared to moderate intensity continuous exercise. Furthermore, the activity of lipoprotein lipase (LPL; a key enzyme involved with the removal of TG) appears to be increased following high intensity interval exercise training (Trombold et al., 2013). This is important because TG clearance appears to be the primary mechanism of reducing postprandial TG after high intensity interval exercise (Burns et al., 2015). Many early exercise interventions designed to reduce postprandial lipids employed moderate intensity continuous exercise and it became widely accepted that estimated energy expenditure was central to these reductions (Freese et al., 2014). However, as reviewed by Burns and colleagues, the estimated energy expenditure during high intensity interval exercise interventions that reduce postprandial TG appear to be lower than that during moderate intensity exercise interventions (Burns et al., 2015). Additionally, when estimated energy expenditure during exercise is matched, high intensity interval exercise has been shown to have a greater effect on reducing postprandial TG (Trombold et al., 2013).

One mechanism by which high intensity interval exercise elicits greater reductions in postprandial TG compared to moderate intensity continuous exercise could be explained by the regulation of LPL and its specificity to type 2 muscle fibres (Burns et al., 2015). A greater number of type 2 muscle fibres will likely be recruited during high intensity interval exercise and subsequently type 2 muscle fibre specific LPL activity may be

greatly increased (Trombold et al., 2013). Reductions in postprandial TG with moderate intensity continuous exercise may also occur via this mechanism because type 2 muscle fibre recruitment increases with prolonged moderate intensity exercise. Exercise duration and energy expenditure for moderate intensity exercise are closely related, it is therefore possible that type 2 muscle fibre recruitment during prolonged moderate continuous exercise increases LPL activity in type 2 fibres. Higher energy expenditure may reflect a greater duration of exercise or exercise at higher intensities and thus increased type 2 muscle fibre recruitment. However, as already discussed, accurate assessment of energy expenditure during high intensity interval exercise is challenging and therefore comparison between moderate intensity exercise and high intensity interval exercise with regards to energy expenditure may be misleading. Further mechanistic investigations into the effects of acute high intensity interval exercise induced attenuation in postprandial TG excursions and fibre specific LPL activity would help to identify optimal exercise interventions for those at risk of cardio-metabolic disease.

Our data support the use of submaximal high intensity interval exercise as a training modality to reduce postprandial TG which may favourably modify lipid-related cardiovascular risk in overweight and obese men.

#### 5.4.2 Exercise and postprandial oxidative stress

There were no improvements in markers of oxidative stress with exercise observed within the present study. This could be due to the small sample size within this study and the variability within these markers. These were also secondary outcome measures and therefore the study was not adequately powered to detect differences between interventions for these markers.

There were no changes in postprandial oxLDL concentrations or lipid hydroperoxides with prior acute submaximal high intensity interval exercise. Reduced oxLDL with endurance cycling exercise (70%  $\dot{V}O_2$ max for approximately 47 minutes) performed 16 h before high fat meal ingestion has been previously reported (Jenkins et al., 2011). Compared to the present study, the high fat meal utilised in the study by Jenkins and colleagues (Jenkins et al., 2011) contained approximately 50g more fat. The higher fat intake is likely to have contributed to a larger and prolonged lipaemic response. Higher circulating lipids provides a greater capacity for postprandial LDL oxidation (Graner et al., 2006) and therefore there may have been a greater capacity for reduction in oxLDL with exercise compared to the present study.

A reduction in lipid hydroperoxides with the exercise session performed either immediately before an OFTT, or 1 hour after an OFTT has been demonstrated previously (Clegg et al., 2007, Mc Clean et al., 2007). However, to the author's knowledge the effects of exercise performed 16 h before an OFTT on lipid hydroperoxides, as investigated in the present study protocol, has not been investigated. Of the studies that have investigated the effects of exercise in reducing postprandial oxidative stress, all employed continuous endurance exercise lasting 47 (Jenkins et al., 2011) or 60 (Clegg et al., 2007, Mc Clean et al., 2007) minutes at an intensity of 70%  $\dot{V}O_2$ max (Jenkins et al., 2011), 60% predicted maximum heart rate (Clegg et al., 2007) or 60% maximum heart rate (Mc Clean et al., 2007). The timing of exercise and perhaps the mode of exercise required to reduce oxidative stress may therefore be important.

#### 5.4.3 Strawberry consumption and postprandial TG

In contrast to previous research (Burton-Freeman et al., 2010), strawberry consumption had no effect on TG AUC. Interestingly, TG iAUC was higher with strawberry



consumption than with the placebo. In contrast to the beneficial effects of strawberry consumption on postprandial TG that have been reported previously (Burton-Freeman et al., 2010) the present findings suggest that strawberry consumption had no effect on postprandial TG.

The OFTT had a higher fat content (73g versus 31g) and the carbohydrate content was considerably lower (33g versus 135g) compared to a previous study which demonstrated reduced TG after OFTT with strawberry consumption (Burton-Freeman et al., 2010). Additionally, the OFTT was composed of milk and cream as opposed to typical American breakfast foods. The author proposes that the differences in carbohydrate quantities of the OFTT and the amount of fructose relative to the total carbohydrate content may explain these findings. Approximately 20% of the carbohydrate content of the strawberry OFTT was fructose, with glucose the predominant carbohydrate source in the placebo OFTT (which did not contain fructose). It has been demonstrated previously that an OFTT containing fructose resulted in a higher postprandial TG response compared to the same OFTT when the carbohydrate content was glucose (Chong et al., 2007). It was proposed by Chong and colleagues (2007) (Chong et al., 2007) that the lower insulin response to fructose compared to glucose may explain the greater postprandial TG response. The fructose content in the strawberry OFTT may therefore have contributed to the greater incremental increase in postprandial TG in this study compared to placebo. Given the relatively small fructose contribution to the high total carbohydrate in the test meals of Burton-Freeman and colleagues (Burton-Freeman et al., 2010), the overall effect of fructose on the insulin response was likely minimal in this study. Further, strawberry polyphenols promote increased insulin sensitivity (Edirisinghe et al., 2011). This could potentially stimulate enhanced insulin mediated TG storage in adipose tissue and thus increased TG

clearance from the circulation, when carbohydrate is high as was the case in the study by Burton-Freeman and colleagues (Burton-Freeman et al., 2010).

#### 5.4.4 Strawberry consumption and postprandial oxidative stress

There were no changes in oxLDL or lipid hydroperoxides between groups. Previous studies have demonstrated the benefits of strawberries on reducing postprandial oxLDL after lipid ingestion (Burton-Freeman et al., 2010, Park et al., 2016). The author gave a dose of strawberries (25g Freeze dried strawberries) which is similar to the optimal dose (20g) for lowering postprandial TG identified by Park and colleagues (2016) (Park et al., 2016). The author used a higher fat content and specifically a higher dairy fat content in this OFTT meal compared to that of other studies (Park et al., 2016, Burton-Freeman et al., 2010). Dairy products within the OFTT in the present study may have reduced circulating bioavailability of the strawberry polyphenols because milk proteins and fat may reduce bioavailability of berry polyphenols (Cebeci and Sahin-Yesilcubuk, 2014, Zhang et al., 2013). However, despite the bioavailability of berry polyphenols being lower when combined with milk, this may not necessarily reduce the intestinal-blood transfer of berry polyphenols according to in vitro experiments (Cebeci and Sahin-Yesilcubuk, 2014). Notably, reduced circulating oxLDL and increased circulating strawberry polyphenols have been observed after consumption of a strawberry drink containing milk in humans (Park et al., 2016). It is therefore unclear whether dairy products reduced the bioavailability of strawberry polyphenols and therefore capacity to reduce oxLDL in the present study. Lipid hydroperoxides, which increase during postprandial lipaemia (Clegg et al., 2007, Mc Clean et al., 2007, Natella et al., 2002) are reduced after anthocyanin intake from grapes (Natella et al., 2002). However, a reduction was not observed in the present study which assumed strawberry anthocyanin

intake. As discussed, the potential for reduced bioavailability with dairy products may explain these findings. Differences in the agricultural and preparation processes of the strawberry products could also contribute to the discrepancies between the present study and previous studies (Basu et al., 2014b).

#### 5.4.5 Limitations

Some of the limitations that exist within the present study have already been eluded to in the discussion. A further limitation is that only the evening meal on the day preceding the OFTT was standardised. Therefore, it cannot be completely excluded that food intake 24 hours before the OFTT influenced the postprandial responses. The participants were given strict instructions to abstain from alcohol, caffeine and their adherence to these instructions was trusted. This was the same for restricting physical activity beyond their habitual levels (which were self-reported to be below standard guidelines), however, previous studies have attempted to measure activity levels during this period (Trombold et al., 2013). Additionally, although the abbreviated 4-hour OFTT has been shown to be predictive of the 8 hour time period (Weiss et al., 2008) and is a repeatable test (O'Doherty et al., 2016) it does not allow assessment of clearance of postprandial TG (this is, CMs and their remnants), which may have been beneficial to evaluate.

#### 5.5 Conclusions

Our findings support the use of acute submaximal high intensity interval exercise as an effective intervention to reduce lipoprotein-related cardiovascular risk factors in overweight and obese adult men. This mode of structured exercise could be

incorporated in to lifestyle management of overweight and obese adult males to reduce cardiovascular risk. However, freeze-dried strawberry supplementation within an OFTT containing dairy products did not improve postprandial TG response which may be related to the fructose and total carbohydrate content of the meal. Nevertheless, this is an interesting finding that merits further investigation. The author recommends that future studies: 1. Investigate the role of carbohydrate and polyphenols in reducing postprandial lipaemia and 2. Evaluate the effects of acute submaximal high intensity interval exercise on reducing postprandial lipaemia in dyslipidaemic males and females.

## Chapter 6: General discussion

### 6.1 Main findings

The purpose of this thesis was to evaluate postprandial lipid metabolism in different cohorts and to investigate the effects of acute exercise and dietary interventions on reducing postprandial lipids. This thesis offers novel contributions to the existing literature, demonstrated in part by 2 of the chapters being published in well regarded peer reviewed journals (O'Doherty et al., 2016, O'Doherty et al., 2017). Chapter 3 establishes the repeatability of postprandial responses to an original OFTT. This OFTT was tailored to meet the most recent expert panel statement by Kolovou and colleagues (2011) and was designed specifically for the investigations within this thesis. Few laboratories appear to have demonstrated the repeatability of their OFTT meals, which is surprising given that demonstrating robust, valid and reproducible methods is important for rigorous experimental research.

Whilst it is largely accepted that acute exercise positively modulates the postprandial lipid response there are certain key areas that had either not been adequately addressed in the literature or had not yet been considered prior to this thesis, these are:

1. Whether the variability of lipaemic responses to OFTT with immediate prior exercise could explain the contrasting literature within this area of research.
2. Whether acute exercise attenuates postprandial lipaemia in OT2D, a group shown to be at increased risk of T2D and known to have abnormal lipaemic responses.
3. Whether the increased serum insulin responses to OFTTs with high carbohydrate content contribute to reductions in postprandial lipaemia in OT2D.

4. Whether submaximal high intensity interval exercise is an effective strategy to reduce postprandial lipaemia.
5. Whether the use of polyphenols through strawberry supplementation can complement the widely reported favourable effects of exercise on postprandial lipaemia.

There is a lack of clarity with regards to whether exercise immediately before OFTT reduces postprandial lipaemia due to conflicting findings in the literature (Emerson et al., 2016, Littlefield et al., 2017). This is complicated further by other experimental differences between studies, including; differences in OFTT meal composition, small sample sizes and subsequent power to detect a “true” difference, and the differences in participants recruited between the studies. These observations are apparent in the selected contemporary investigations of acute exercise on postprandial lipaemia outlined in *Table 1.1* of the introduction. Furthermore, the proposed mechanisms associated with reduced postprandial lipaemia such as increased LPL activity are unlikely to occur during this short time frame between acute exercise and subsequent OFTT ingestion (Maraki and Sidossis, 2013). To the author’s knowledge, whilst the reliability or repeatability of OFTTs had been assessed in the resting condition, prior to publication of the study presented in chapter 3, this aspect had not been investigated in the acute exercise condition. This is particularly important given the fairly even distribution of studies that exist showing either an effect (Littlefield et al., 2017, Plaisance et al., 2008, Ferreira et al., 2011, Katsanos and Moffatt, 2004, Katsanos et al., 2004) or those indicating no effect on reducing postprandial TG (Pfeiffer et al., 2005, Petridou et al., 2004, Cox-York et al., 2013, Clegg et al., 2007, Katsanos et al., 2004, Canale et al., 2014). This investigation demonstrated considerable variability in the effect of immediate prior exercise on postprandial lipaemia. This variability could therefore explain the different outcomes reported in the literature and the results of this

study should encourage careful consideration for future studies seeking to reduce postprandial lipaemia with acute exercise performed shortly before OFTT meal ingestion. However, there are notable differences between this study and some of the previous studies that could potentially influence outcomes, such as; the higher exercise intensities employed compared to the present study and the different composition of OFTTs, particularly the higher carbohydrate content. Therefore, the repeatability of the effect of acute prior exercise under these conditions could also be evaluated in future studies. The implications of the findings in this reproducibility study for the subsequent studies reported within this thesis were as follows;

1. The specifically designed OFTT provided reliable measures of postprandial responses in normolipidaemic, healthy, adult males. This OFTT could therefore be used with confidence for the interventional studies that followed.
2. The beneficial effects of immediate prior exercise on postprandial lipaemia are not clear. From a practical logistic perspective, exercising participants immediately before an OFTT is useful as it reduces study visits compared to exercising participants on the evening before OFTTs. However, due to the considerably larger evidence base for reduced postprandial lipaemia when exercise is performed the evening before OFTT (Plaisance and Fisher, 2014, Maraki and Sidossis, 2013), the timing of the exercise was given further consideration and moved to the evening before OFTT for the intervention studies that followed in this thesis.

The variability of carbohydrate content in OFTT meals across studies assessing postprandial lipid responses is interesting. The OFTT is designed to assess postprandial lipid responses after a moderate (30g to 50g) or high (50g to 80g) amount of fat (Lopez-Miranda et al., 2007). However, carbohydrate (excluding fructose) considerably

increases secretion of insulin; a master regulator of both glucose and lipids (Pirillo et al., 2014, Ginsberg et al., 2005). Of note, the quantities of carbohydrate provided within OFTTs are often in excess of the 75g provided in oral glucose tolerance tests (see *Table 1.1*), designed to assess postprandial glucose regulation (Matsuda and DeFronzo, 1999). As such these OFTTs challenge both postprandial carbohydrate and fat metabolism and this should be considered within the study design. Raised circulating insulin concentrations reduce hepatic VLDL secretion and consequential rises in postprandial TGs in insulin sensitive participants (Malmstrom et al., 1998) but not insulin resistant participants (Kriketos et al., 2005). Inadequate suppression of hepatic VLDL secretion and impaired insulin mediated clearance of circulating glucose at the muscle, leads to increased de novo lipogenesis. In insulin resistant individuals, this accounts for up to a 60% rise in circulating postprandial TG (Petersen et al., 2007). These mechanisms may explain why Kriketos et al. (2005) identified a reduction in postprandial TG in lean healthy participants but not OT2D after HFHC compared to HFLC OFTTs.

The aims of the second experimental study of this thesis, Chapter 4, were to replicate and extend on the above cited findings in OT2D by Kriketos and colleagues (2005), by evaluating whether acute moderate intensity exercise could favourably modify these postprandial responses. This is important because of the strong relationship between increased circulating postprandial TG and cardio-metabolic disease, particularly in adults already at an increased risk of cardio-metabolic disease (Boren et al., 2014, Wang et al., 2016, Varbo et al., 2013b). Furthermore, the design of this study allowed evaluation of postprandial responses with high and low circulating insulin concentrations (in response to high or low carbohydrate OFTTs) and with the OFTT preceded by rest and a prior acute exercise session. This was considered an important question because the mechanisms underlying the beneficial effects of exercise on postprandial lipid metabolism are not fully understood. Improvements in insulin



sensitivity appears a plausible explanation for improved lipid metabolism with insulin resistant individuals and is supported by a recent mechanistic study (Rabøl et al., 2011). However, this mechanism was dismissed previously, based on indirect evidence (Gill et al., 2002). The feasibility study presented in this thesis showed large effect sizes for lower postprandial TG AUC and iAUC in the acute exercise and high carbohydrate OFTT condition. These findings must be interpreted with caution until they have been replicated in a large adequately powered study. Nonetheless, these findings indicate that prior acute exercise and high circulating insulin concentrations may interact to reduce postprandial TG in OT2D. These findings support the importance of regular exercise in these higher risk participants and provides mechanistic insight in to the role of acute exercise and related insulin sensitivity in improving postprandial TG responses. In addition, the utilisation of imaging techniques to quantify skeletal muscle and liver fat and glycogen content, using natural isotopes to trace fat metabolism in muscle and liver, similar to techniques observed in studies by Petersen et al. (2007) and Rabøl et al. (2011), would complement the present study design.

A notable finding from the study presented in Chapter 4 was the slow recruitment to the trial of volunteer OT2D, despite using various advertising methods. The OFTT (beyond the standard or even non-fasting lipid profile) is most relevant in those who are sedentary, overweight/obese and have clustered risk factors for cardio-metabolic disease. Given the time frame of the PhD process, it was decided that overweight/obese, sedentary adult males would be selected as a higher risk apparently healthy cohort for the final study (Chapter 5).

During the PhD process a review evaluating the effects of HIIE on reducing postprandial lipids was published from the Stensel laboratory (Burns et al., 2015). This review highlighted the beneficial effects of HIIE on postprandial lipids. However, it was

noted that there was a paucity of data on submaximal HIIE interventions. The previous study (chapter 4) incorporated moderate intensity continuous exercise with a fairly low energy expenditure compared to most, but not all studies within the literature (Lopes Kruger et al., 2016, Emerson et al., 2016). Although the underlying mechanisms of exercise in reducing postprandial lipids are not entirely clear, HIIE is an effective strategy to complete a larger volume of exercise within a shorter period of time than continuous moderate intensity exercise (Little et al., 2010). This provides the potential to elicit a greater amount of energy expended and greater activation of type 2 muscle fibres (which may be relevant for increased LPL activity (Hamilton et al., 1998)) compared to moderate intensity exercise. Given the above observations and the contemporary interest in this exercise modality, HIIE was selected for the final study. HIIE was found to be an effective strategy for reducing postprandial TG 4-hour AUC (by approximately 20%) in overweight/obese healthy male participants. Therefore, this mode of exercise may be an effective preventative strategy for treating postprandial dyslipidaemia and lowering cardio-metabolic disease risk in overweight and obese males.

Nutritional interventions using polyphenol rich foods have been shown to lower lipid oxidation, a key proponent of atherosclerotic risk in the postprandial period (Burton-Freeman et al., 2010, Edirisinghe et al., 2011). The proposed mechanisms for nutritional modification of postprandial oxidative stress appear to be different from the exercise mediated reductions. Therefore, acute supplementation of polyphenols within mixed meal challenges, in combination with prior acute exercise may be synergistic to reducing postprandial cardio-metabolic risk factors. Conversely, contrary to previous literature, the final experimental study in this thesis (Chapter 5) did not find a beneficial effect of acute polyphenol supplementation with freeze dried strawberries. Moreover, there was a significant effect for increased TG iAUC with acute strawberry intake.

There are plausible explanations for this finding. Firstly the OFTT was composed of milk and cream which may have reduced the capacity for absorption of polyphenols. Second, the author did not match fructose content across trials, oral ingestion of fructose does not stimulate increased insulin concentrations in the same manner as other carbohydrates (Chong et al., 2007). Chapter 4 highlights the potential importance of insulin responses to OFTTs in regulating circulating glucose and lipid concentrations. Accordingly, the relationship between acute polyphenol supplementation (through strawberries or alternative polyphenol rich fruits) and their independent and combined effects with acute exercise interventions merit further evaluation.

## 6.2 Future directions

The future directions of this work include; an adequately powered multi-centre study investigating the effects of acute exercise on postprandial responses to OFTTs (incorporating both HFLC and HFHC) in OT2D would be required to substantiate the preliminary findings observed in chapter 4. This investigation could be supported with the use of advanced technologies (MRS with labelled isotopes) to help to identify underlying mechanisms linking the interventions to specific changes in postprandial responses. The findings in chapter 5 related to acute strawberry intake contrast with previous literature (Burton-Freeman et al., 2010, Edirisinghe et al., 2011). The purported benefits of strawberry intake merits further investigation. In this respect, some of the differences in this study meal compared to the previous literature have been eluded to. A non-dairy, high fat intervention is recommended for future investigations. The promising acute effects of HIIE on measures of insulin sensitivity and postprandial lipid metabolism in overweight/obese men provides an opening for future studies. Chronic exercise interventions using this protocol could be investigated to assess cardio-metabolic health benefits. Finally, these studies were only performed in adult

male participants and these should also be repeated in adult female participants to assess whether the same benefits of prior exercise on postprandial lipaemia are transferrable to women.

### 6.3 Strengths and limitations

The strengths and limitations of the studies have been considered in each of the respective study chapters. There are, however, some considerations that are common to all studies and these will be highlighted in this section. The strengths of these studies include the robust crossover experimental study designs included. The crossover design that was used throughout this thesis meant that each participant served as their own control, strengthening the confidence in the findings. The OFTT meals that were employed throughout the studies were specifically designed to keep consistent with an expert panel statement on postprandial testing for cardio-metabolic risk stratification (Kolovou et al., 2011). The meal given to each participant on the evening before the OFTT was standardised, this prior meal has been shown in previous studies to impact upon the postprandial lipid response (Robertson et al., 2002). Every aspect of data collection (i.e. making the OFTT, blood collection, CPET interpretation, protocol design, preparation and storage of blood, amongst others) during each study was performed by one person (Alasdair O'Doherty), this improves consistency with the way that the studies are performed. Participants were asked not to exercise on the day before the OFTT (excluding the exercise session for the experimental protocol). The use of activity monitors during this intervening time would have been useful to confirm that participants performed the same amount of activity on each day prior to the OFTT.

Due to the number of study visits and the apparent inconsistency of responses to lipid metabolism after OFTTs in females (Gill et al., 2005), this series of studies only

recruited adult male participants. Therefore, further studies with female participants are required to confirm whether the findings of this thesis are applicable to adult females.

#### 6.4 Personal development and reflection

Throughout the PhD journey I have learned and developed as an academic and a person by both building upon existing knowledge and experiences and having new experiences. I gained skills in repeatability, feasibility and fully powered experimental trials. I have built upon my previous experiences in writing NHS ethics to gain successful ethical approval from all of the studies within this thesis. My clinical skills and knowledge base also helped me to overturn a faculty health and safety policy that did not allow cannulation to be performed within the department. I demonstrated my resourceful skills by re-commissioning a biochemistry analyser that had been redundant within the department. I have developed a wider appreciation for statistical methods, being faced with the challenges of assessing repeatability in non-normally distributed data. I shared knowledge through presentations at scientific conferences and with the local patient group for people living with type 2 diabetes and patients living with cardiovascular disease. I learned new biochemistry techniques that allowed me to analyse all of my blood samples (excluding insulin). I built upon my previous experiences in scientific paper writing skills and the peer review process by publishing two peer reviewed articles. I built upon my previous grant writing skills and achieved a small grant as the lead applicant from a local charity. I am grateful for the opportunities that I have had throughout the PhD process and believe that the whole experience has prepared me for a career in academia.

## 6.5 Conclusions

The findings of this thesis indicate that the 4-hour abbreviated OFTT is a reliable test to assess postprandial lipid metabolism. However, the abbreviated 4-hour OFTT was not reproducible when moderate intensity exercise is performed immediately before the OFTT. Following the preliminary findings of this thesis, the role of an acute exercise session in improving insulin sensitivity as a mechanism for improving postprandial TGs should be reconsidered and further evaluated, especially in those with insulin resistance at increased risk of cardio-metabolic disease. Further consideration should also be given to the carbohydrate content within OFTT meals, particularly when utilised as an outcome measure within interventional studies. Finally, submaximal HIIE appears to be effective in attenuating postprandial TG when performed the evening before an OFTT in apparently healthy overweight men. However, contrary to existing literature, acute strawberry intake with an OFTT was not effective in reducing the adverse lipid related perturbations that occur in the postprandial period. These findings add novel information to the existing literature on several aspects of acute exercise and postprandial lipid metabolism. The included studies have offered an explanation for conflicting literature in the area of acute exercise interventions. Notably, this thesis has provided some data on the underlying mechanisms for postprandial responses in insulin resistant states that have been largely unchallenged or disregarded. Finally, this thesis highlights the potential health benefits of moderate and submaximal HIIE for reducing postprandial cardio-metabolic risk in young and middle-aged, overweight, sedentary adult males. Adopting these exercise strategies as part of daily lifestyle routines may therefore reduce cardio-metabolic risk in these cohorts.

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## Appendix 1: Ethical approval for chapter 3

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<b>Independent reviewer's name</b>	Dr Andrew Garrett
<b>Application number</b>	1415063
<b>Principal investigator's name</b>	Professor Sean Carroll
<b>Student investigator's name (if applicable)</b>	Alasdair Doherty

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## The repeatability of the abbreviated (4-h) Oral Fat Tolerance Test and influence of prior acute aerobic exercise

A. F. O'Doherty<sup>1</sup> · T. Sathyapalan<sup>2</sup> · A. S. Rigby<sup>3</sup> · L. Ingle<sup>1</sup> · S. Carroll<sup>1</sup>Received: 30 June 2016 / Accepted: 29 September 2016  
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### Abstract

**Purpose** The Oral Fat Tolerance Test (OFTT) is regarded as a repeatable measure used to assess postprandial triglyceride (TAG) levels, with higher levels observed in cardio-metabolic disorders. Acute aerobic exercise intervention before OFTT reduces the TAG response, but the repeatability of this effect is unknown. The aim of this study was to determine the repeatability of the abbreviated 4-h OFTT with and without immediate prior aerobic exercise.

**Methods** On four separate days, healthy adult male participants underwent two 4-h OFTT ( $n = 10$ ) and another two 4-h OFTT with 1-h of standardised moderate intensity aerobic exercise performed immediately before meal ingestion ( $n = 11$ ). The OFTT meal composition included 75.4 g total fat, 21.7 g carbohydrate and 13.7 g protein. Venous blood was sampled at baseline and hourly up to 4 h after the OFTT meal ingestion, and TAG area under the curve (AUC) was calculated.

**Results** Nonparametric Bland–Altman analysis of 4-h TAG AUC revealed that 9 of 10 repeat measurements fell within  $\pm 15\%$  of the median TAG AUC for the OFTT. By contrast, two of 11 repeat measurements fell within  $\pm 15\%$  of the median TAG AUC for the OFTT undertaken with 1-h prior aerobic exercise.

**Conclusions** The 4-h OFTT is a repeatable test of postprandial TAG responses in healthy men. However, aerobic exercise performed immediately before OFTT considerably increases the variability of TAG AUC. These findings have implications for interpretation of research studies investigating exercise intervention performed immediately before OFTT. Future studies should also investigate the repeatability of exercise performed 8–24 h before OFTT.

**Keywords** OFTT · Postprandial metabolism · Acute exercise · Lipids · Repeatability

### Introduction

The Oral Fat Tolerance Test (OFTT) is used to assess the capacity to adapt postprandial metabolic processes after a predefined oral fat load and evaluate cardio-metabolic health [1–5]. After oral fat consumption ( $>15$  g), triglyceride (TAG) levels rise in the blood, typically peaking at 3–4 h and returning to baseline 6–8 h later [6]. These rises are exacerbated in those with cardiovascular and metabolic disorders and are associated with progression of atherosclerosis [1, 6, 7]. Since humans spend most of the day in the postprandial state, OFTT may reveal cardio-metabolic dysfunction not detected by traditional fasting measures [6, 8, 9].

At present, the OFTT is not widely used in a clinical setting to assess cardio-metabolic function. One reason for this could be the time demands of OFTT [10] which typically require postprandial measurements to be taken every 1 or 2 h for 5–8 h [11–16]. However, recently an abbreviated OFTT (lasting 4-h) has been developed and validated [3, 10]. This test would reduce the time constraints, improve the practicality of OFTT [10] and has been recommended by an expert consensus as clinically relevant

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Independent reviewer's name	Dr Andrew Garrett
Application number and title	1415205 The effect of prior exercise on postprandial responses to high fat meals with high or low carbohydrate content in offspring of people with type 2 diabetes
Principal investigator's name	Professor Lee Ingle
Student investigator's name (if applicable)	Alasdair O Doherty

Reviewer's recommended outcome			
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Reviewers comments	
Section	Comment
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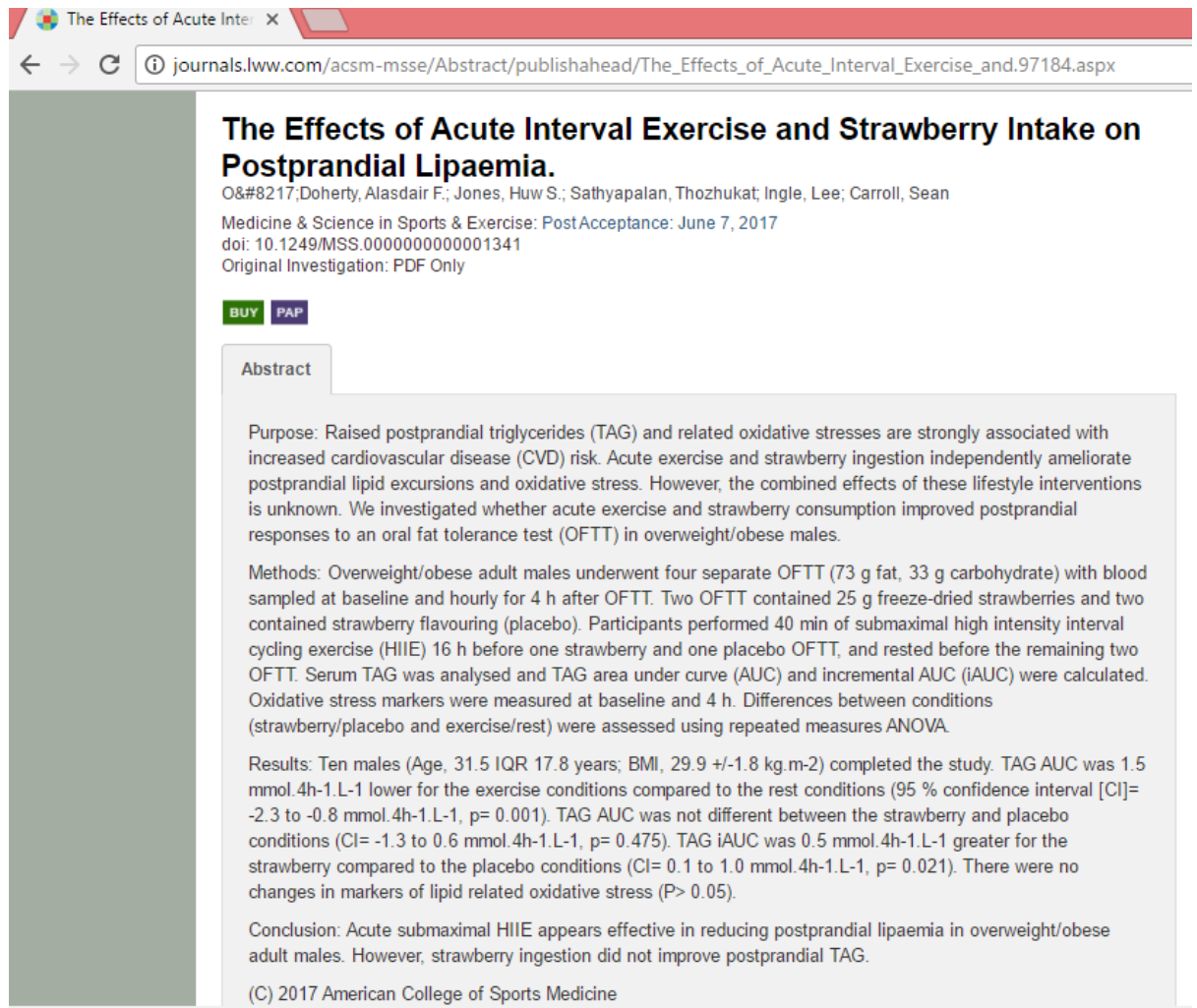
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Independent reviewer's name	Dr Andrew Garrett
Application number and title	Exercise and Strawberry consumption to acutely reduce postprandial cardiovascular and metabolic impairment after lipid ingestion 1516094
Principal investigator's name	Professor Sean Carroll
Student investigator's name (if applicable)	Alasdair O Doherty

<b>Reviewer's recommended outcome</b>			
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Reviewers comments	
Section	Comment
Click here to enter text.	This study is suitable for ethics approval on the basis the cannulation is carried out by an approved experimenter on the staff approval lists held by Prof. Lee Ingle. In this instance it will be the applicant (Alistdair O Doherty).
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Appendix 5: Publication of chapter 5 in Medicine and Science in Sports and  
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### The Effects of Acute Interval Exercise and Strawberry Intake on Postprandial Lipaemia.

O&#8217;Doherty, Alasdair F.; Jones, Huw S.; Sathyapalan, Thozhukat; Ingle, Lee; Carroll, Sean

Medicine & Science in Sports & Exercise: PostAcceptance: June 7, 2017  
doi: 10.1249/MSS.0000000000001341  
Original Investigation: PDF Only

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**Abstract**

**Purpose:** Raised postprandial triglycerides (TAG) and related oxidative stresses are strongly associated with increased cardiovascular disease (CVD) risk. Acute exercise and strawberry ingestion independently ameliorate postprandial lipid excursions and oxidative stress. However, the combined effects of these lifestyle interventions is unknown. We investigated whether acute exercise and strawberry consumption improved postprandial responses to an oral fat tolerance test (OFTT) in overweight/obese males.

**Methods:** Overweight/obese adult males underwent four separate OFTT (73 g fat, 33 g carbohydrate) with blood sampled at baseline and hourly for 4 h after OFTT. Two OFTT contained 25 g freeze-dried strawberries and two contained strawberry flavouring (placebo). Participants performed 40 min of submaximal high intensity interval cycling exercise (HIIE) 16 h before one strawberry and one placebo OFTT, and rested before the remaining two OFTT. Serum TAG was analysed and TAG area under curve (AUC) and incremental AUC (iAUC) were calculated. Oxidative stress markers were measured at baseline and 4 h. Differences between conditions (strawberry/placebo and exercise/rest) were assessed using repeated measures ANOVA.

**Results:** Ten males (Age, 31.5 IQR 17.8 years; BMI, 29.9 +/-1.8 kg.m<sup>-2</sup>) completed the study. TAG AUC was 1.5 mmol.4h-1.L-1 lower for the exercise conditions compared to the rest conditions (95 % confidence interval [CI]= -2.3 to -0.8 mmol.4h-1.L-1, p= 0.001). TAG AUC was not different between the strawberry and placebo conditions (CI= -1.3 to 0.6 mmol.4h-1.L-1, p= 0.475). TAG iAUC was 0.5 mmol.4h-1.L-1 greater for the strawberry compared to the placebo conditions (CI= 0.1 to 1.0 mmol.4h-1.L-1, p= 0.021). There were no changes in markers of lipid related oxidative stress (P> 0.05).

**Conclusion:** Acute submaximal HIIE appears effective in reducing postprandial lipaemia in overweight/obese adult males. However, strawberry ingestion did not improve postprandial TAG.

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