University of Nottingham
UK | CHINA | MALAYSIA

# Chronic effects of high intensity interval training on postprandial lipaemia in healthy individuals.

Andrew Wilhelmsen BSc (Hons)

Thesis submitted to the University of Nottingham for the degree of Master of Research, Physiology.

September 2017

Supervisor: Dr Kostas Tsintzas

Co-supervisor: Professor Paul Greenhaff

# Abstract

**BACKGROUND:** Dyslipidaemia is associated with atherosclerosis and subsequent cardiovascular disease. Acute high intensity interval exercise (HIIT) is a time-efficient means to improve postprandial lipaemia (PPL), but little is known about its cumulative effects. **AIM:** To investigate the chronic effects of HIIT on PPL and metabolic health in healthy young individuals. **METHODS:** Following ethical approval, 8 recreationally active males (mean ± SD: 22 ± 3years, 1.77 ± 0.07m, 67.7 ± 6.2kg) undertook two 6h mixed-meal tolerance tests, before the first session and ≥72h after the final session of four weeks of HIIT (16 sessions; 10x60s bouts of cycling at 90%$VO_{2max,}$ interspersed by 60s intervals at 45%$VO_{2max}$). A *vastus lateralis* muscle sample was taken in the fasted state before and after training. Regular arterialised and deep venous blood samples across the forearm, brachial artery blood flow measurements, and whole body indirect calorimetry data were obtained before, and at regular intervals for 6h after consumption of the mixed-meal tolerance test before and after training. **RESULTS:** $VO_{2max}$ (mean ± SEM) increased from 47.30 ± 5.46 to 51.80 ± 3.41m$LO_2 \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.01$), without changes in body mass. Neither fasting circulating triglyceride (TAG) concentrations and postprandial responses, nor skeletal muscle protein content of lipolytic enzymes were altered with training. Free fatty acid (FFA) forearm uptake and fractional extraction significantly increased after training (ANOVA main effects; $P = 0.03$ and $P = 0.048$, respectively), with a strong trend towards increased blood flow in the latter half of the tolerance test ($P = 0.07$). **CONCLUSIONS:** Four weeks of HIIT increases cardiovascular fitness and the postprandial uptake and extraction of FFA across the forearm but has no chronic effect on circulating TAG. Our findings suggest the frequently observed exercise-induced reduction in postprandial TAG is a transient effect of the last exercise bout, and highlight the importance of regular exercise for the maintenance of training-induced benefits to postprandial lipaemia.

# Acknowledgements

It would be unjust to submit this thesis without first expressing my gratitude to Dr Kostas Tsintzas. His support throughout the past 12 months has been exceptional; his advice and guidance invaluable; and his enthusiasm unwavering. Having not originally planned to undertake a Masters by Research, it is with Kostas' encouragement that I have come to realise how greatly valuable the experience has been, and I am grateful to have had the opportunity.

To Dr Joanne Mallinson, I am also indebted for the training she provided; her involvement in the running of the study; for her willingness to help wherever possible; and for her relentlessly positive attitude. Many thanks are also due to Professor Paul Greenhaff for his support in designing the study. Lastly, my sincerest thanks to Sara Brown, the doctors, technicians, fellows, PhD students and all those who helped to organise, supported, offered advice and provided technical assistance, for collectively enabling the successful completion of this project.

# Declaration

This study has been primarily self-funded, with a contribution from the University of Nottingham's School of Life Sciences. The study design and ethical approval process was carried out by Dr Kostas Tsintzas, Professor Paul Greenhaff, and myself. Recruitment, screening, cardiopulmonary exercise testing and exercise sessions were carried out exclusively by myself. On main study visit days, cannulations and muscle biopsies were performed by Dr Tariq Taylor, Dr Natalie Shur or Dr Lee Creedon, while deep venous blood samples, blood flow, and muscle thickness measurements were performed by Dr Joanne Mallinson. All other main study visit duties were carried out by myself. Assessment of plasma free fatty acids, serum insulin and triglycerides were carried out by Sally Cordon. All other laboratory work was completed by myself under the support and guidance of Dr Kostas Tsintzas, Dr Scott Cooper, and Mr Robert Jones.

I hereby declare that, apart from the aforementioned, all work undertaken and presented in this thesis is the product of my own efforts, under the supervision of Dr Kostas Tsintzas, during my time enrolled as a student at the University of Nottingham. All sources of information, products and procedures, and visual artwork are duly referenced and acknowledged as accurately as possible. No part of this thesis has been submitted in any form for another higher degree.

Signed: _____

Andrew Wilhelmsen

Date: _____

# Table of Contents

Abstract ..... I

Acknowledgements ..... II

Declaration ..... III

List of Figures and Tables ..... VII

Abbreviations ..... X

Chapter 1: Literature Review ..... 1

1.1 Atherosclerosis ..... 1

1.1.1 Risk Factors and Rate of Occurrence ..... 3

1.1 Lipids and Lipoproteins ..... 3

1.2.1 Triglycerides ..... 3

1.2.2 Cholesterol ..... 4

1.2.3 Phospholipids ..... 4

1.2.4 Lipoproteins ..... 4

1.2.6 Chylomicrons ..... 6

1.2 Lipaemia ..... 6

1.3.1 Fasting Lipaemia ..... 6

1.3.2 Postprandial Lipaemia ..... 7

1.4 Acute exercise and Fasting Lipaemia ..... 8

1.4.1 Continuous Exercise ..... 8

1.4.2 High Intensity Interval Training ..... 10

1.5 Acute Exercise and Postprandial Lipaemia ..... 11

1.5.1 Continuous Exercise ..... 11

1.5.2 High Intensity Interval Training ..... 13

1.5.3 Gender Differences ..... 15

1.6 Chronic Exercise and Fasting Lipaemia ..... 16

1.6.1 Continuous Exercise ..... 16

1.6.2 High Intensity Interval Training ..... 17
1.7 Chronic Exercise and Postprandial Lipaemia ..... 19
1.7.1 Continuous Exercise ..... 19
1.7.2 High Intensity Interval Training ..... 20
1.8 Aims ..... 23
Chapter 2: Methods ..... 24
2.1 Study design ..... 24
2.2 General Proceedings ..... 24
2.3 Participants ..... 25
2.4 Cardiopulmonary Exercise Testing ..... 25
2.5 Dietary and Physical Activity Standardization ..... 26
2.6 Main Visit Proceedings ..... 26
2.6.1 Bergstrom Muscle Biopsy ..... 27
2.6.2 Tolerance Test Meal ..... 28
2.6.3 Blood Sampling ..... 28
2.6.4 Blood Flow and Substrate Uptake ..... 29
2.6.5 Muscle Thickness ..... 30
2.6.6 Indirect Calorimetry ..... 31
2.7 HIIT Protocol ..... 33
2.8 Sample Analysis ..... 33
2.8.1 Muscle Protein Extraction and Quantification ..... 33
2.8.2 Western Blotting ..... 33
2.8.3 Circulating Triglyceride ..... 34
2.8.4 Circulating Free Fatty Acids ..... 35
2.8.5 Circulating Insulin ..... 35
2.8.6 Branched Chain Amino Acids ..... 36
2.9 Statistical Analysis ..... 36
Chapter 3: Results ..... 38

3.1 Characteristics, Completion and Standardisation ..... 38
3.2 Cardiopulmonary and Musculoskeletal Responses ..... 40
3.3 Resting Substrate Oxidation and Energy Expenditure ..... 43
3.4 Blood Biomarkers and Peripheral Blood Flow ..... 44
3.5 Protein Content of Lipolytic Enzymes in Skeletal Muscle ..... 54
Chapter 4: Discussion ..... 56
4.1 Overview of Findings ..... 56
4.2 Lipid and Lipolytic Enzyme Responses ..... 56
4.3 Brachial Artery and Blood Flow Responses ..... 61
4.4 Blood Glucose and Insulin Responses ..... 63
4.5 Circulating Branched Chain Amino Acid Responses ..... 66
4.6 Resting Substrate Oxidation and Energy Expenditure ..... 68
4.7 Cardiopulmonary Responses ..... 69
4.8 Hypertrophic Response to Training ..... 69
4.9 Limitations ..... 70
Chapter 5: Conclusions ..... 72
5.1 Summary ..... 72
5.2 Future Directions ..... 73
References ..... 74

# List of Figures and Tables

Figure 1. Progression of atherosclerosis from the initial accumulation of lipoproteins (A) and their migration into the vessel wall (B); the formation of foam cells (C) and their development into a necrotic core (D); to the rupturing of the plaque; thrombus formation; and subsequent downstream blood flow restriction (E). Adapted from Servier Medical Art. ..... 2

Figure 2. Lipoprotein classifications and structural components (not to scale). Adapted from Servier Medical Art. ..... 5

Figure 3. Schematic diagram of the structure of the study. ..... 24

Figure 4. Schematic diagram of the main study visit, which was undertaken pre- and post-training intervention. ..... 26

Figure 5. Bergstrom biopsy needle comprising an outer cannula with a small opening near the tip (A), an inner trocar with a cutting blade (B) and a pushing rod to expel collected tissue (C). ..... 27

Figure 6. Ultrasound image from the determination of brachial artery diameter (A) and flow velocity (B) in a representative subject. ..... 29

Figure 7. Ultrasound image of the vastus lateralis muscle (at 50% of femur length) in a representative subject with muscle thickness measurement highlighted (solid line between aponeuroses). ..... 31

Figure 8. Three-day energy intake and macronutrient contribution prior to the baseline and post-intervention main visits. Data determined from self-reported diet diaries using Nutritics dietary analysis software. Data displayed as mean ± SEM; n=8. ..... 39

Figure 9. Mean (vertical bars) and individual (horizontal lines) changes in maximal oxygen uptake ($VO_{2max}$) before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8 (A). The relationship between baseline $VO_{2max}$ and percent change $VO_{2max}$, quantified using Pearson's product moment, after 16 sessions of HIIT; n=8 (B). Maximal oxygen uptake significantly increased with training, **P < 0.01. Baseline $VO_{2max}$ negatively correlated with $\Delta VO_{2max}$, $^{\dagger\dagger}P < 0.01$. ..... 41

Figure 10. Changes in muscle thickness of the rectus femoris (RF), the medial and lateral portions of the vastus intermedius (VI), and vastus lateralis (VL), of both the right and left leg, measured at 50% of femur length via B-mode ultrasonography, before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=4. Muscle thickness of the RD and VL significantly increased with training, **P < 0.01..... 42

Figure 11. Resting rates of carbohydrate (A) and fat oxidation (B), respiratory exchange ratio (C) and metabolic rate (D) prior to and 2, 4 and 6h post meal challenge, before (baseline) and after 16 sessions of HIIT (post). Oxidation rates are corrected for protein oxidation via equations derived from Frayn (1983), using urine and plasma urea concentrations. Resting metabolic rates are determined using the Weir equation (1949). Data displayed as mean ± SEM; n=8..... 44

Figure 12. Fasted (t=0) and postprandial blood flow through the brachial artery, assessed via Doppler ultrasonography before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8. In the final 3h of the tolerance test, brachial artery blood flow tended to increase after training, ‡P = 0.07 (ANOVA main effect). ..... 48

Figure 13. Fasted (t=0) and postprandial glucose concentrations (A and B), arterio-venous difference (C) and subsequent rate of uptake, before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8. ..... 49

Figure 14. Fasted (t=0) and postprandial plasma insulin concentrations (A), HOMA2 insulin resistance (B) and Matsuda Index (derived from insulin and glucose concentrations during fasting and 0-120min after feeding), before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8..... 50

Figure 15. Fasted (t=0) and postprandial triglyceride concentrations (A and B), arterio-venous differences (C) and subsequent rate of uptake, before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8. ..... 51

Figure 16. Fasted (t=0) and postprandial free fatty acid concentrations (A and B), arterio-venous differences (C), subsequent rate of uptake (D) and fractional extraction (E), before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8. Forearm free fatty acid uptake and extraction significantly increased after training, $^{\#}P < 0.05$ (ANOVA main effect)..... 52

Figure 17. Fasted (t=0) and postprandial branched chain amino acid concentrations (A and B), arterio-venous differences (C) and rate of uptake (D), before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8..... 53

Figure 18. Lipoprotein lipase protein content (normalised against cyclophilin B) in vastus lateralis muscle before (baseline) and after 16 sessions of HIIT (post). Muscle samples taken from fasted, resting participants following ≥72h abstinence from exercise. Data displayed as mean ± SEM; n=6. Top: bands of LPL protein at ~52kDa and CypB protein at ~19kDa, at baseline and post-training, in 2 representative participants. ..... 54

Figure 19. Adipose triglyceride lipase protein content (normalised against cyclophilin B) in vastus lateralis muscle before (baseline) and after 16 sessions of HIIT (post). Muscle samples taken from fasted, resting participants following ≥72h abstinence from exercise. Data displayed as mean ± SEM; n=6. Top: bands of ATGL protein at ~55kDa and CypB protein at ~19kDa, at baseline and post-training, in 2 representative participants..... 55

Table 1. Participant physical characteristics before (baseline) and after 16 sessions of HIIT (post). ..... 38

Table 2. Pre-training (baseline) and Post-training (post) fasted, resting values of blood biomarkers and brachial artery characteristics. ..... 45

# Abbreviations

Adipose Triglyceride Lipase.....ATGL

Aerobic Interval Training.....AIT

Analysis of Variance.....ANOVA

Apolipoprotein.....Apo

Area Under the Curve.....AUC

Brachial Artery Diameter.....BAD

Branched-Chain Amino Acid.....BCAA

Branched-Chain Oxoacid Dehydrogenase.....BCOAD

Carbohydrate.....CHO

Cardiovascular Disease.....CVD

Cyclophilin B.....CypB

Flow Mediated Dilation.....FMD

Free Fatty Acid.....FFA

Glyceraldehyde 3-Phosphate.....G3P

High Intensity Interval Training.....HIIT

High-Density Lipoprotein.....HDL

Homeostatic Model Assessment 2 of Insulin Resistance.....HOMA2-IR

Hormone Sensitive Lipase.....HSL

Hydrogen Peroxide.....$H_2O_2$

Incremental Area Under the Curve.....iAUC

Insulin Receptor Substrate 1 .....IRS-1

Intermediate-Density Lipoprotein.....IDL

International Physical Activity Questionnaire.....IPAQ

Intramuscular Triglyceride.....IMTG

Lipoprotein Lipase.....LPL

Low-Density Lipoprotein.....LDL

Maximal Oxygen Uptake.....$VO_{2max}$

Mean Difference.....MD

Messenger Ribose Nucleic Acid.....mRNA

Milli-, Micro-, Nano-mole.....mmol, µmol, nmol

Moderate Intensity Continuous Training.....MICT

Molar.....M

Nicotinamide Adenine Dinucleotide.....NAD

Oral Fat Tolerance Test.....OFTT

Oral Glucose Tolerance Test.....OGTT

Postprandial Lipaemia.....PPL

Respiratory Exchange Ratio.....RER

Resting Metabolic Rate.....RMR

Sodium Dodecyl Sulphate.....SDS

Sprint Interval Training.....SIT

Standard Deviation.....SD

Standard Error of the Mean.....SEM

Total Area Under the Curve.....tAUC

Triglyceride (triacylglyceride, triacylglycerol) .....TAG

Tris Buffered Saline – Tween 20.....TBS-T

Very-Low-Density Lipoprotein.....VLDL

# Chapter 1:
# Literature Review

## 1.1 Atherosclerosis

Atherosclerosis is a complex disease that arises from a plethora of lifestyle factors and individual genetic profile, which is associated with subsequent negative cardiovascular outcomes including peripheral arterial and coronary heart disease (Kulbertus & Lancellotti, 2012). Atherosclerosis has been defined as a focal inflammatory, fibro-proliferative response to multiple forms of endothelial injury, beginning in the early teens with intimal lesions in the form of fatty streaks (George & Johnson, 2010; Insull, 2009; Strong et al., 1999).

Exposure of the arterial endothelium to elevations of low-density lipoprotein (LDL), free radicals and shear stress, can cause accumulation of lipoprotein particles which trigger an innate immune response that recruits and activates inflammatory cells (Davis, 2005; Gustafsson & Boren, 2004). Chemokines, interleukins and proteases increase the permeability of the endothelium to monocytes and lymphocytes (Fig. 1; A) (Tedgui & Mallat, 2006). Leukocytes migrate deeper into the vessel walls, where oxidation and non-enzymatic glycation products initiate local inflammation (Fig. 1; B) (Tonkin, 2003). Pro-inflammatory cytokines augment the expression of endothelial adhesion molecules and are involved in the production of growth factors which further compromise the vessel wall (Barath et al., 1990; Boyle, 2005; Mendis, Puska, & Norrving, 2011).

As the lesion evolves, monocytes engulf modified lipoprotein particles within the vessel wall; differentiate into macrophages; and form lipid-laden foam cells (Fig. 1; C) (Galkina & Ley, 2009). Smooth muscle cells migrate from the media to the intima, where they proliferate and lay extracellular matrix forming a collagenous cap (sclerosis) over the lesion (Katsuda & Kaji, 2003). Inflammatory cytokines cause programmed cell death

(apoptosis) of trapped monocytes and macrophages, forming a necrotic core (Fig. 1; D) (Ghosh, Zhao, Bie, & Song, 2010).

Remodelling of the arterial wall allows some plaque growth with minimal blood flow restriction (Armstrong, Heistad, Marcus, Megan, & Piegors, 1985; Williams, Armstrong, & Heistad, 1988). Eventually, reparations become futile as leukocytes and lipid fragments enter the lesion from its weaker lateral aspects (Davis, 2005). Plaques can rupture, expelling lipid fragments and cellular debris into the lumen, where thrombogenic agents initiate thrombus (blood clot) formation (Fig.1; E). Development within a coronary artery may cause myocardial infarction (heart attack), while formation in the cerebral vasculature can cause ischaemic stroke (Mendis et al., 2011).

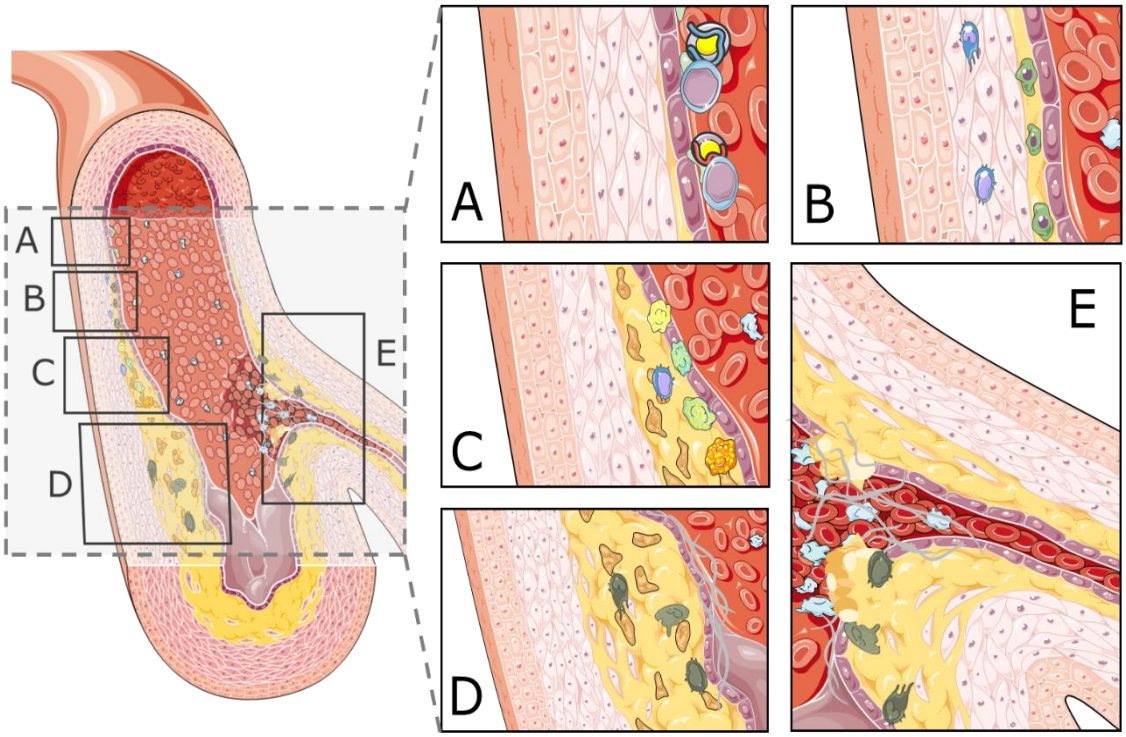


**Figure 1.** Progression of atherosclerosis from the initial accumulation of lipoproteins (A) and their migration into the vessel wall (B); the formation of foam cells (C) and their development into a necrotic core (D); to the rupturing of the plaque; thrombus formation; and subsequent downstream blood flow restriction (E). Adapted from Servier Medical Art.

### 1.1.1 Risk Factors and Rate of Occurrence

For many years it was widely agreed that atherosclerosis was ascribable to elevated concentrations of serum cholesterol; believed to be the product of high dietary fat content (Stamler, 1978). More recently it has been suggested that high levels of small, dense LDL (common in diets high in saturated and trans fatty acids), have deleterious effects on the vascular wall (Despres et al., 1990). Conversely, high-density lipoprotein (HDL) rich diets may be atheroprotective by removing cholesterol from foam cells for shuttling or excretion (reverse cholesterol transport) (Badimon & Vilahur, 2012).

Arterial wall thickness and luminal cross-sectional area of elastic arteries increases with age, even in the absence of atherosclerotic plaques (Gardner & Parker, 2010; Lakatta, 1993; Nagai et al., 1998; Vaitkevicius et al., 1993). While the probability of atherosclerosis-related mortality in middle aged men in the United Kingdom has declined from 22% in 1950 to just 6% in 2010, greater life expectancy means more people are living with the disease (Herrington, Lacey, Sherliker, Armitage, & Lewington, 2016; Vos et al., 2015).

## 1.1 Lipids and Lipoproteins

Lipids serve as a high density fuel source, structural building block, covalent attachment for molecular guidance, and as intracellular messengers (Berg, Tymoczko, & Stryer, 2012). In western diets, fats typically contribute 30-35% of energy intake (Austin, Ogden, & Hill, 2011; Vadiveloo, Scott, Quatromoni, Jacques, & Parekh, 2014).

### 1.2.1 Triglycerides

Triglycerides (TAG) are the most physiologically abundant form of lipids, forming ~90% of dietary fats (Athenstaedt, 2010). Exogenous TAG is hydrolysed in the small intestine, forming free fatty acids (FFA) and monoglycerides (MAG), which form aggregates called micelles that passively diffuse across enterocyte membranes and are re-esterified to

TAG (Smith & Morton, 2001). Endogenously, increased hepatic FFA delivery (a product of fat feeding, or fasted lipolysis) drives re-esterification of FFA and monoglyceride to TAG through the action of insulin.

### 1.2.2 Cholesterol

Cholesterol is the precursor of all steroid hormones and its dysregulation results in various disease states (Cortes et al., 2014; Jousilahti et al., 1998). Cholesterol is acquired from the diet, but primarily by endogenous synthesis. Phytosterols, found exclusively in plants, closely resemble cholesterol, but may acutely lower LDL by displacing cholesterol from micelles (Gylling & Simonen, 2015).

### 1.2.3 Phospholipids

Obtained from dietary intake of egg yolk, soy, milk and marine sources, phospholipids are a major cell membrane component comprising a glycerol backbone esterified to two fatty acids and a phosphate head group.

### 1.2.4 Lipoproteins

Lipoproteins are globular particles of high molecular weight, responsible for lipid transport. Lipoproteins comprise a phospholipid and cholesterol monolayer membrane embedded with apolipoproteins (apo; lipid binding proteins, responsible for receptor and enzyme recognition) encompassing a core of TAG and cholesterol esters (Leray, 2014). Lipoproteins are commonly classified as chylomicrons or very-low-, intermediate-, low- or high-density (Fig. 2).

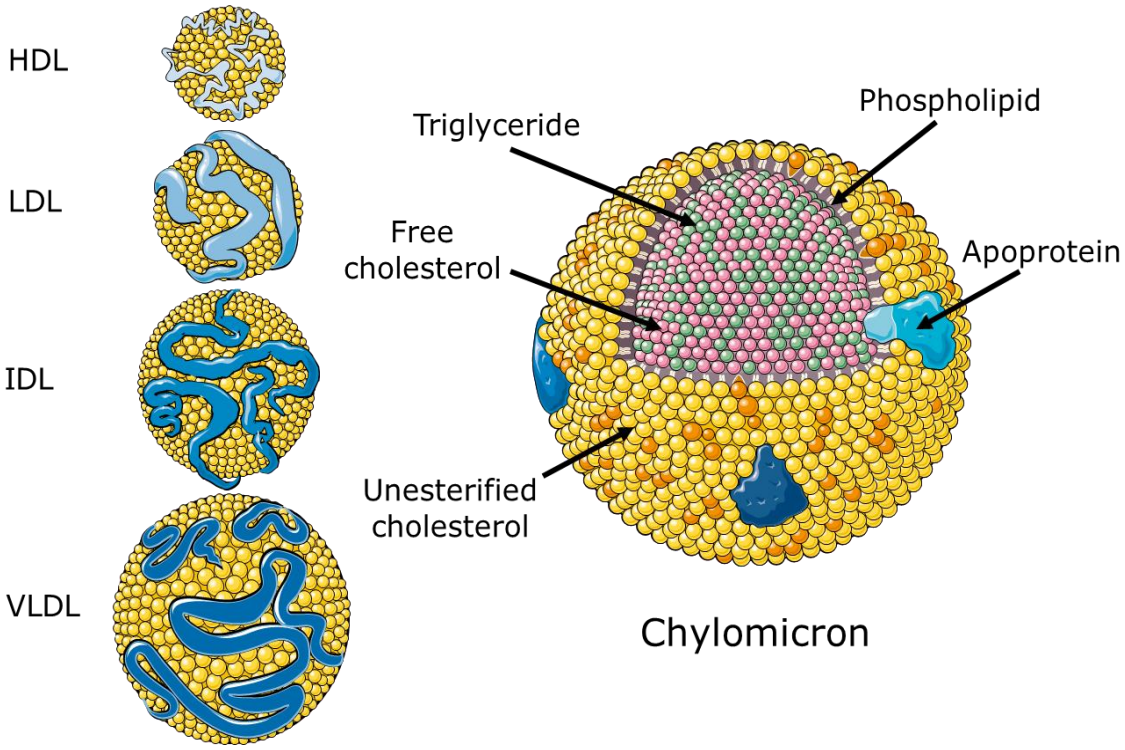


**Figure 2.** Lipoprotein classifications and structural components (not to scale). Adapted from Servier Medical Art.

Very-low-density lipoproteins (VLDL) are the primary vehicle for endogenous TAG transport, which comprise ~55% of their contents by weight (Leray, 2014). Primordial VLDL fuse with apoC-II and apoE from HDL, forming mature VLDL. Lipoprotein lipase (LPL) removes TAG from VLDLs, while cholesteryl-ester transfer protein mediates cholesterol transport from HDL to VLDL in exchange for phospholipids and TAG, transforming VLDL to IDL (Goldberg, Scheraldi, Yacoub, Saxena, & Bisgaier, 1990; Lagrost et al., 1993; Morton, 1999).

Half of IDLs are rapidly cleared from plasma to the liver (Golan & Tashjian, 2012). Hepatic lipase hydrolyses TAG in the remaining IDLs, making them denser and cholesterol ester-enriched, while ApoE and apoC-II are transferred to HDL due to reduced affinity. Once cholesterol exceeds TAG content, IDL are classified as LDL; the principle transporter of cholesterol in plasma (Feingold & Grunfeld, 2000).

Derived from VLDL and chylomicrons, HDL comprise ~55% proteins and ~25% phospholipids (Leray, 2014). Often referred to as 'good cholesterol', HDL plays a key role in the transport of cholesterol from peripheral tissues to the liver (Gordon, Castelli, Hjortland, Kannel, & Dawber, 1977; G. J. Miller & Miller, 1975; Whayne, 2009).

### 1.2.6 Chylomicrons

Chylomicrons, the largest and least dense of all lipoproteins, are involved in the transport of exogenous TAG, cholesterol, and fat-soluble vitamins to peripheral tissues and the liver. Lipids, primarily TAG, constitute ~99% of chylomicron mass, and their size is related to recent fat ingestion (Cohn et al., 1993; Hussain, Kedees, Singh, Athar, & Jamali, 2001; K. W. Miller & Small, 1983). Proteins, present as apoB48, apoAIV, apoAI, and apoC's, constitute ~1% by mass, but play essential roles in assembly, secretion and recognition. Lipoprotein lipase plays a key role in the catabolism of chylomicrons. Hydrolysis depletes ≤85% of chylomicron TAG, forming smaller, cholesterol-enriched particles, termed remnants, which return bile cholesterol to the liver (Havel, 1998; Redgrave, 2004). Macrophages can uptake remnants, which may induce TAG and cholesterol accumulation; promoting atherosclerosis (Botham, 2008).

## 1.2 Lipaemia

The nomenclature surrounding dyslipidaemia is non-standardised. Generally, lipaemia refers to the state of normal blood lipid concentrations, while hyperlipaemia refers to their abnormally high concentrations. Approximately 25% of adults in the US are estimated to exhibit elevated fasting TAG ($\geq 1.7 mmol \cdot L^{-1}$), which often occurs with secondary disorders including type II diabetes mellitus and the metabolic syndrome (Carroll, Kit, & Lacher, 2015; Hegele et al., 2014).

### 1.3.1 Fasting Lipaemia

Hokanson and Austin (1996) reported that a $1.0 mmol \cdot L^{-1}$ increase in fasting TAG was associated with a 14% elevation in cardiovascular disease

(CVD) risk after adjustment for covariates. At mild-to-moderately raised TAG concentrations (2.0-10.0mmol·$L^{-1}$), lipoproteins are typically small enough to enter the arterial wall and accumulate, progressing atherosclerosis (Nordestgaard & Varbo, 2014). However, in subjects with chylomicronaemia syndrome and extremely high fasting TAG (>25mmol·$L^{-1}$), premature atherosclerosis remains a low risk, likely because 50-70% of their lipoproteins are too large to cross endothelial cells (Nordestgaard, Stender, & Kjeldsen, 1988; Nordestgaard & Zilversmit, 1988). Subsequently, fasting measurements may only be a suitable method of risk assessment in healthy individuals.

### 1.3.2 Postprandial Lipaemia

Elevated non-fasting TAG, remnant cholesterol and TAG-rich lipoproteins are independent risk factors for CVD and all-cause mortality, but only non-fasting TAG remains predictive once total and HDL cholesterol, and insulin resistance are accounted for (Bansal et al., 2007; Nordestgaard & Varbo, 2014). Since much of waking time in western populations is spent in the postprandial state, heightened postprandial lipaemia (PPL) is a cause for concern. Perez-Martinez (2016) suggests oral fat tolerance tests (OFTT) may offer more useful information for individuals with fasting TAG between 1.0-2.0mmol·$L^{-1}$; reporting approximately half of such subjects to exhibit 'hidden' postprandial hyperlipaemia.

It has been suggested that day-long TAG profiling, with natural feeding habits in 'free-living' scenarios offers highly applicable results that are closely related to those of fat loading tests (Castro Cabezas, Halkes, Meijssen, van Oostrom, & Erkelens, 2001; van Wijk, Cabezas, Halkes, & Erkelens, 2001). While this may offer greatest real-life relevance, it lacks standardization, limiting applicability to whole populations. Furthermore, dietary intake can often only be feasibly monitored with food diaries, which are susceptible to under-reporting, especially in obese subjects (Goris, Westerterp-Plantenga, & Westerterp, 2000; van Wijk et al., 2001).

Regular time-point OFTTs are most commonly utilised for PPL assessment. Unlike the clinically-routine oral glucose tolerance test (OGTT) which

utilises a standard load of 75g anhydrous glucose (British National Formulary, 2014), no standardised composition nor sampling protocol has been adopted. This methodological inconsistency is a limiting factor for the comparison of results.

It has been recommended that a test meal should provide between 70-80g of fat comprising a mixture of saturated and unsaturated fats, 25g carbohydrate (CHO) and 10g protein to ensure a full range of metabolic responses (Kolovou et al., 2011). This quantity was associated with the highest mean difference in peak TAG values compared with fasting and is a comparable load to the self-reported daily fat intake of ~65g in US males (Vadiveloo et al., 2014). Lairon (2007), however, suggest that 50-100g of CHO should be included to ensure the elicitation of insulin-dependent postprandial processing of dietary TAG. The addition of protein to the meal appears to mitigate the lipaemic response, however the mechanisms underpinning this interaction remain largely un-investigated (Westphal et al., 2006). Mixed liquid-solid meals better represent normal eating behaviour, gastric emptying/absorption rates, and thus metabolic and hormonal responses during the test (Lairon et al., 2007).

In healthy individuals, postprandial TAG responses produce a bell-shaped curve, typically peaking at 4h before returning to baseline within 5-8h (Dubois et al., 1998; Nordestgaard, Benn, Schnohr, & Tybjaerg-Hansen, 2007). Abbreviated 4h assessments have demonstrated high reproducibility and predicted 8h responses ($R^2$ = 0.89-0.96), but longer time-courses remain preferable (Weiss, Fields, Mittendorfer, Haverkort, & Klein, 2008).

## 1.4 Acute exercise and Fasting Lipaemia

### 1.4.1 Continuous Exercise

Most traditional exercise modalities utilise a continuous workload, velocity or intensity, which is maintained for an extended period of time, often termed moderate intensity continuous training (MICT). In the first few hours following MICT, fasting TAG concentrations are typically unchanged

from those of the pre-exercised state. Some studies have reported elevated levels of TAG after prolonged exercise (3-4 h), possibly due to an exercise-induced increase in lipolysis, and consequent increase in FFA hepatic delivery (Dufaux, Order, Muller, & Hollmann, 1986; Thompson, Cullinane, Henderson, & Herbert, 1980).

The day after continuous exercise, fasting measures of lipaemia are almost universally reduced irrespective of exercise intensity, providing sufficient energy is expended. Even exercise intensities as low as 31% $VO_{2max}$ have been shown to reduce fasting TAG if performed for 2-3 hours (Aldred, Perry, & Hardman, 1994; Tsetsonis & Hardman, 1996).

Acute MICT appears to reduce lipaemia dose-dependently, suggesting TAG-reduction is the product of an exercise-induced energy deficit (Gill, Herd, & Hardman, 2002; Peddie, Rehrer, & Perry, 2012). Annuzzi and colleagues (1987) demonstrated that doubling exercise duration, while maintaining a fixed intensity (1.5h vs. 3h, at 77% $HR_{max}$), doubled the reduction in fasting TAG (17% vs. 33%, respectively). Consistent with these changes, a 23% fasting TAG reduction was achieved 18h after a 2h treadmill walk at 50% $VO_{2max}$ (Gill, Herd, Vora, & Hardman, 2003). Contrastingly, following one or two hours of cycling, Magkos et al. (2007; 2006) did not demonstrate the same apparent linear relationship. Instead, 55% and 65%, respective increases in FFA appearance were described. Further, neither protocol reduced VLDL-TAG concentration, however a 40% increase in TAG clearance rate was observed following the longer duration exercise.

It has been suggested that exercise alters the hepatic partitioning of long-chain FFA between esterification and oxidation, lowering hepatic TAG secretion (Fukuda, Tojho, Hidaka, Sho, & Sugano, 1991). Restricted VLDL secretion lowers circulating TAG concentration, while decreasing competition for LPL-mediated hydrolysis (Westphal, Orth, Ambrosch, Osmundsen, & Luley, 2000). Indeed, β-hydroxybutyrate, a marker of hepatic fatty acid oxidation, has been shown to increase concurrently to

lowering TAG, after 90min of treadmill walking (Gabriel, Ratkevicius, Gray, Frenneaux, & Gray, 2012; Gill et al., 2007).

When exercise-induced energy deficits are compared to dietary interventions (Diet group intake = Exercise group intake – Exercise energy expenditure), equivalent caloric deficits do not offer the same degree of postprandial TAG reduction (Gill & Hardman, 2000). It appears that exercise uniquely stimulates some factor(s) that reduce the rate of appearance, and/or increase clearance of TAG-rich lipoprotein particles in the postprandial window (Burns, Miyashita, & Stensel, 2015). Furthermore, these changes may occur chronically in the absence of weight loss, suggesting that exercise interventions may be superior in reducing lipaemia than energy restriction alone (Kelley, Kelley, & Vu Tran, 2005).

### 1.4.2 High Intensity Interval Training

High intensity interval training (HIIT) utilises repeated bouts of short (typically 30–240s), intense exercise (>85% $VO_{2max}$), followed by an equal or longer period of recovery (Plaisance & Fisher, 2014). Such protocols have grown popular in the last decade, favoured for their time-efficiency and greater perceived level of enjoyment, which may improve adherence (Aamot, Karlsen, Dalen, & Stoylen, 2016; Bartlett et al., 2011; Smith-Ryan, 2015).

The recent embracing of HIIT and the adoption of PPL assessment has resulted in little research interest into the effects of HIIT on fasting measures. Many postprandial studies, however, take fasting measures prior to the provision of a meal. A single bout of HIIT appears to elicit similar responses to those of MICT, with significantly lower energy expenditure. Bellou (2013) reported a 20% reduction in fasting plasma VLDL-TAG following high volume HIIT (4x4;4min bouts of 90% and 60% $VO_{2max}$). Thackray, Barrett and Tolfrey (2013) found a small to moderate difference (MD=-0.05mmol·$L^{-1}$, ES=0.4) in fasting plasma TAG ~16h after 10x60s bouts of running at maximal aerobic speed, with by 60s recovery intervals. In a low volume HIIT study, in which participants undertook 4x30s all-out cycling sprints, Freese and colleagues (2011) reported a

trend towards lowered fasting TAG (~20% reduction, $P$ = 0.07). The authors also found a 10% reduction in postprandial TAG total area under the curve (tAUC).

## 1.5 Acute Exercise and Postprandial Lipaemia

### 1.5.1 Continuous Exercise

The postprandial effects of continuous exercise vary greatly with different exercise modalities, intensities, durations, and time between exercise and assessment. Thirty minutes of MICT (90% gas exchange threshold) immediately before high-fat feeding appears to increase flow mediated dilation (FMD) of the brachial artery, but does not influence postprandial TAG levels 1-3h after exercise (Bond, Gates, et al., 2015). Using an identical exercise protocol 1h prior to feeding, TAG incremental area under the curve (iAUC) was unaffected in boys, but was reduced by 38% in girls (Bond, Williams, et al., 2015). In contrast, ~50min of MICT (60 – 70% $VO_{2max}$) 1h pre-feeding has been shown to reduce TAG iAUC by 32% in middle aged males presenting with the metabolic syndrome (Plaisance et al., 2008).

Much of the literature supports some degree of dose-responsiveness with regards to exercise-induced energy expenditure and PPL in a ~12-24h post-exercise window. Freese and colleague's (2014) review reported a moderate to low correlation (r = -0.31) between energy expenditure and Cohen's *d* for aerobic exercise. This dose-dependency has been challenged by an absence of difference in postprandial TAG reduction after 60 minutes of moderate versus vigorous running, despite 45% greater energy expenditure in the latter (Tolfrey et al., 2008). Furthermore, just 30min walking at 60% HRmax has been shown to reduce TAG AUC, the following day, by 18% in healthy Japanese males (M. Miyashita et al., 2013).

Several recent meta-analyses attempted to determine threshold values for energy expenditure and exercise intensity, when energy balance is maintained. Maraki and Sidossis (2010) identified a required exercise energy expenditure of 30kJ·kg$^{-1}$ (~7.2kcal·kg$^{-1}$); equal to 2.25MJ for a

75kg individual. They later concluded that ≥2.0-2.5MJ (≥500-600kcal) was required to produce meaningful effects in healthy populations (likely less in the obese), equal to ~90min of brisk walking or cycling (Maraki & Sidossis, 2013). Similarly, Teeman et al. (2016) reported that acute exercise from 18h prior to, until 90min post-feeding, can attenuate PPL. It was suggested that the exercise should be ~60min duration at a moderate intensity. As might be expected, lower expenditure appears to be required for adolescents to elicit similar responses. Tolfrey and colleagues (2014) established in their review that ≥1.0MJ (~240kcal) expenditure reliably lowers postprandial TAG in boys and girls, 12-18h after exercise.

With a view to improve applicability to populations with limited time or exercise-capacity, recent research has investigated the effects accumulating short bouts of physical activity throughout the day, or combining exercise with dietary energy restriction. Maraki and colleagues (2009) demonstrated that the combination of 100min of light walking (30% $VO_{2max}$; expending ~1MJ) and 1.39MJ of energy restriction on the day of exercise, resulted in an 18% reduction in fasting TAG the following day. It has been established that exercise-induced energy expenditure, not the nature or pattern of that exercise, is the most important factor for lowering PPL (Masashi Miyashita, Burns, & Stensel, 2013). When spread across the day, 10x3min bouts of brisk walking or running, comparably mitigate postprandial TAG to a single 30min bout of matched exercise (M. Miyashita, Burns, & Stensel, 2006; Masashi Miyashita, Burns, & Stensel, 2008).

The efficacy and clinical significance of accumulating physical activity on postprandial metabolism was recently highlighted by Hensen et al. (2016). In a study of postmenopausal women with impaired glucose regulation, compared to 7h of unbroken sitting, 5min of standing or light walking every 30min improved same-day postprandial responses of FFA, glucose and insulin, but not TAG, by 20-47%. Responses were not different between the standing and walking interspersions, however when reassessed the following day, both interventions sustained glucose iAUC reduction, but only walking maintained the suppression of insulin.

### 1.5.2 High Intensity Interval Training

While energy expenditure appears to play a central role in determining PPL following MICT, emerging research suggests that HIIT may offer comparable or greater outcomes with lower expenditure (Plaisance & Fisher, 2014). Indeed, in a comprehensive review of literature, Freese et al. (2015) found no significant correlation between HIIT energy expenditure and Cohen's *d*, and suggested that the greater intensity of contractile activity may alter lipid metabolism without the need for great expenditure. Significant reductions in postprandial TAG have been observed following a single bout of HIIT, with energy expenditures as low as ~100kcal (Gabriel et al., 2013; Gabriel et al., 2012). Interestingly, TAG and insulin AUC reductions following isoenergetic MICT and HIIT (500kcal expenditure) were not significantly different in a study of healthy young males (Ferreira et al., 2011).

High-intensity interval training can broadly be divided into two sub-categories; 'supramaximal' and 'submaximal'. Typically, submaximal or 'near-maximal' HIIT utilises intensities ≥80% $HR_{max}$, while supramaximal or 'all-out' training is any workload ≥100% $VO_{2max}$, sometimes termed sprint-interval training (SIT) (MacInnis & Gibala, 2016). Some studies have employed higher volume protocols at lower intensities, typically just above the 'anaerobic threshold' (Ferreira et al., 2011; Tyldum et al., 2009). Consideration must be given to such studies, since US guidelines classify 'hard' physical activity as 65-85% $VO_{2max}$, however it may be dubious to appropriate them as HIIT as they better reflect MICT intensities. Accordingly, we shall instead employ the term 'aerobic interval training' (AIT).

Many early studies on muscle performance and enzymatic adaptations to HIIT employed 4-7 bouts of 30s all-out cycling separated by ~5min recovery (Jacobs, Esbjornsson, Sylven, Holm, & Jansson, 1987; MacDougall et al., 1998). The effectiveness of the protocol has translated to its regular employment in studies of PPL. Reductions in TAG AUC of between 10 and 21% the following day have been reported when energy

expenditure was not replaced with refeeding (Freese et al., 2011; Gabriel et al., 2013) and ~10% when recompensed (Freese et al., 2011). Tan et al. (2013) did not observe any such reduction using identical exercise and a similar tolerance meal protocol. The authors also found no change in TAG AUC following 20min MICT at 70% $VO_{2max}$, which contradicts some of the literature. Of note, diet was not standardised and participants were not fasted, which may have resulted in hyper-compensatory energy intake. Further, capillary blood samples were employed to assess TAG levels using a dry-chemistry analyser, which cannot detect TAG concentrations <0.8mmol·$L^{-1}$, and may have reduced the accuracy of results.

Unlike findings described previously with MICT, TAG reduction after 5x30s sprints may not be associated with increased β-hydroxybutyrate, suggesting that HIIT-induced postprandial TAG reduction is not a product of reduced hepatic VLDL secretion (Gabriel et al., 2013). Discordantly, β-hydroxybutyrate has been reported to be equally elevated and TAG iAUC reduced after MICT and isoenergetic HIIT (2min intervals at 90% and 25% $VO_{2max}$), compared to a non-exercising control group, but research on the matter remains sparse (Trombold, Christmas, Machin, Kim, & Coyle, 2013).

A small number of studies have considered the effects of extremely short duration sprint interval training (SIT), performing 20-60 x 6-8s cycling sprints. Twenty sprints of 6s duration, interspersed with 24s rest intervals, did not alter TAG tAUC in young males (Allen et al., 2014), whereas 40 and 60 sprints of 8s each (with 90s and 12s recovery intervals, respectively) both resulted in a 13% reduction in TAG AUC in young males, females and adolescent boys (Sedgwick, Morris, Nevill, & Barrett, 2015; M. Tan, Chan Moy Fat, Boutcher, & Boutcher, 2014). This suggests a minimum threshold requirement of ~320s maximal effort to lower PPL, which despite similar intensity, is substantially greater than the 120s of work performed in the 4x30s HIIT model.

Of growing popularity is a 10x60s at ~90% $HR_{max}$ protocol with 60s rest intervals, developed by Gibala et al. (2010). The resultant protocol offers

a training scenario that can be achieved outside of a physiology laboratory (Hood, Little, Tarnopolsky, Myslik, & Gibala, 2011), while retaining a high degree of muscle fibre recruitment within a short duration compared to MICT. A single bout of this HIIT has been shown to reduce time spent in hyperglycaemia by 40% in type II diabetics; improve markers of muscle oxidative capacity (i.e. citrate synthase activity, cytochrome *c* oxidase subunit IV (COX IV), and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) protein content by ~20-70% in both sedentary adults and type II diabetics (Gillen et al., 2012; Hood et al., 2011; Little et al., 2011; Little, Jung, Wright, Wright, & Manders, 2014).

Researchers at Loughborough University utilised the 10x60s protocol to investigate its potential for lowering PPL, reporting moderately lower TAG concentration and TAG AUC ~20h after the HIIT session (effect size = 0.50 and 0.58, respectively, with a ~10% reduction in TAG AUC) (Thackray et al., 2013). Subsequent work suggest the findings to be robust, with highly similar results observed in adolescent girls (Thackray, Barrett, & Tolfrey, 2016).

### 1.5.3 Gender Differences

Few studies have investigated responsive differences between sexes, with women having not been greatly studied. In a review, Freese (2015) reported sex to be a significant moderator of the acute effect of exercise on PPL, with females exhibiting a greater reduction in total TAG than males (Cohen's *d* = -0.96 and -0.57, respectively). Only 16 of a total 121 effects assessed by the review included female participants, however the magnitude of the difference between effect sizes suggests a disparity between the sexes which warrants further investigation.

While Cox-York and colleagues (2013) did not find a significant TAG-lowering effect of exercise in males or females, the authors did report that females, overall, had a 34% lower TAG iAUC. In a study of sedentary men, VLDL-TAG was found to be reduced by 20% 14h post-HIIT, which was attributed to a 21% increase in VLDL-TAG clearance rate but not secretion (Bellou, Magkos, et al., 2013). In women, however, an almost identical

increase in VLDL-TAG clearance rate (+22%) was accompanied by a 17% reduction in hepatic VLDL-TAG secretion rate (Bellou, Siopi, et al., 2013). Similarly, Sondergaard et al. (2011) observed reduced VLDL-TAG secretion during and after 90 minutes of MICT cycling in a fasted state.

## 1.6 Chronic Exercise and Fasting Lipaemia

There exists an abundance of evidence highlighting the beneficial role of physical activity in the reduction of key biomarkers associated with atherosclerosis and CVD (Palmefors, DuttaRoy, Rundqvist, & Borjesson, 2014). Despite this, almost half of men and women in England fail to meet government guidelines, with diminishing attainment with age (Townsend, Wickramasinghe, Williams, Bhatnagar, & Rayner, 2015). 'Lack of time' remains the most frequently cited reason for physical inactivity, suggesting a demand for shorter duration forms of exercise (Townsend et al., 2015).

The seminal work of Holloszy and Booth (1976) revealed adaptations to chronic exercise including changes in mitochondrial function; substrate utilisation; and muscle fibre properties. To date, far less is understood about the effects of exercise training on the regulation of circulating lipids and lipoproteins; further research into which could aid personalised prevention and management strategies for lipid disorders and CVD (Trejo-Gutierrez & Fletcher, 2007).

### 1.6.1 Continuous Exercise

Early research identified that endurance trained individuals typically exhibited lower fasting TAG than their untrained counterparts (Merrill et al., 1989). It was assumed that this represented some physiological adaptation(s) to exercise training. Various research groups went on to investigate this by assessing fasting TAG pre- and post-intervention.

Early studies often implemented insufficient periods of 'washout' or failed to standardise this time-course (Peddie et al., 2012). Aldred, Hardman and Taylor (1995) sought to evaluate the influence of 12 weeks of brisk walking on lipaemia in sedentary middle-aged women, but ceased training ≤48h

prior to assessment. After brief exercise abstinence, the authors observed only subtle changes in serum lipids and lipoproteins, and no effect on insulin or glucose, in the fasted state. PPL was also assessed, revealing no differences in peak TAG, or TAG AUC, but did report a 35% reduction in serum insulin, though this may be partly attributable to ~1kg loss of body mass across the study. Similarly, following 48h of exercise abstinence, habitually trained individuals did not exhibit different fasting or postprandial TAG concentrations to their untrained counterparts. However, the insulinaemic response of trained males was 29% lower than that of untrained (Herd et al., 2000). Older findings suggesting chronic TAG-lowering adaptations with MICT have since been largely refuted as the remnant effects of a the last exercise bout (Maraki & Sidossis, 2013).

Perhaps the most insightful paper in this field is that of Kelley et al. (2005), who undertook a meta-analysis of randomised controlled trials of ≥8-week aerobic exercise interventions on fasting lipaemia in overweight and obese adults. Thirteen studies were included, comprising some 613 subjects, with up to 17 measured outcomes. Random-effects modelling returned significant improvements in TAG (11%), HDL (3%) and total cholesterol (2%), however only TAG remained significant once sensitivity analyses were performed. None of the included studies assessed lipaemia >72h after the final bout of exercise. One study allowed ≤72h for post-intervention testing, reporting ~12% lower fasting TAG in the training versus control group after 6 weeks of supervised exercise. This change was diminished when exercise was maintained without supervision (Ligtenberg, Hoekstra, Bol, Zonderland, & Erkelens, 1997).

### 1.6.2 High Intensity Interval Training

High intensity interval training appears to offer comparable, and possibly greater, physiological adaptations previously associated with MICT. As little as 2 weeks of HIIT has been shown to confer improvements in muscle oxidative capacity, reduce glycogen utilisation and lactate production, and to increase protein content of glucose transporter 4 (GLUT4) and PGC-1α (a 'master regulator' of mitochondrial biogenesis) (K. A. Burgomaster et

al., 2007; K. A. Burgomaster, Heigenhauser, & Gibala, 2006; Kirsten A. Burgomaster et al., 2008; K. A. Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005). It seems reasonable to speculate that similar responses in fasting lipids and lipoproteins might be yielded through regular HIIT, however consideration must be given to the possible transiency of results.

Interestingly, Babraj et al. (2009) reported no changes in fasting glucose or insulin concentrations after 2 weeks of HIIT, however a trend towards reduced plasma FFA concentration was noted (~17% reduction, $P = 0.06$). However, when performed thrice weekly for 8 weeks (4x4;4min running at 90% and 60% $VO_{2max}$, respectively) without weight-loss or compositional changes, Tsekouras (2008) reported an acute ~28% reduction of fasting plasma VLDL-TAG concentration. This reduction was accredited to a ~35% reduction in hepatic VLDL-TAG secretion rate 48h post-exercise. It is difficult to determine whether such outcomes are a transient product of prior exercise or a chronic adaptation. Following the $5^{th}$ of 5 exercise bouts in 5 consecutive days, elevations in LPL mRNA and protein levels peaked at 4h and 8h post-exercise respectively, and were shown to have returned to baseline 20h after the $4^{th}$ bout (Seip, Mair, Cole, & Semenkovich, 1997). Since elevated LPL activity is heavily implicated in, though not the sole moderator of, exercise-induced reductions in lipaemia, it can be postulated that chronic HIIT may confer additional changes in lipid metabolism within the 72h window. This may be in part due to a prolonged or heightened increase in LPL activity with HIIT compared to MICT, however this has been contested (Peddie et al., 2012).

FMD has been reported to stay elevated for 1 but not 3 days in the fasted state (Bond, Cockcroft, et al., 2015). Importantly however, in the same study, postprandial FMD was found to be elevated after 3 days, having not decreased below that of 1-day post-training intervention. This further highlights the importance of simultaneously assessing multiple markers in both the fasting and postprandial state, to determine the effects and potential mechanisms of exercise training on lipaemia.

## 1.7 Chronic Exercise and Postprandial Lipaemia

### 1.7.1 Continuous Exercise

Much like fasting TAG studies, early research purported to have demonstrated, in many populations, that regular exercisers experienced significantly lower PPL than the untrained (Ericsson et al., 1982; Podl et al., 1994; Sady, Cullinane, Saritelli, Bernier, & Thompson, 1988). Once more, however, failure to provide sufficient time between the final exercise session and the OFTT means that it is not possible to distinguish between transient and enduring effects.

While Drexel (1992) did report a sustained reduction in postprandial TAG concentration four days after the last exercise bout, participants lost over 4kg during the course of the training intervention. Weight-loss without exercise training has a known propensity to reduce some, though not all, markers of lipaemia (Dallongeville et al., 2007; A. P. James et al., 2003). Meta-analysis of 50 applicable studies indicated that despite equal weight-loss between HIIT and MICT studies, HIIT produces greater reductions in insulin resistance (Jelleyman et al., 2015). Furthermore, changes in insulin resistance were not associated with body mass reduction, suggesting HIIT independently stimulates factors responsible for enhanced control of circulating glucose, though its effect on lipids is less clear.

It is probable that a multitude of mechanisms are responsible for the reductions in lipaemia typically observed after interventional studies, of which, weight-loss and subsequently improved insulin sensitivity may be central (Maraki, Aggelopoulou, Christodoulou, Anastasiou, et al., 2011). Some studies suggest weight-loss is non-essential for such responses. Again, conclusions are limited by short durations between the last exercise bout and assessment. Six months of walk training, without weight-loss or re-composition, improved insulin sensitivity, hepatic- and lipoprotein-lipase activities (changes in the latter were inversely correlated with those of the total:HDL ratio), despite no changes in fasting lipids ≤48h after the final exercise session (Duncan et al., 2003). Weintraub et al. (1989)

reported significantly reduced fasting and peak TAG and tAUC of the chylomicron fraction of retinyl palmitate (indicative of intestinally derived lipoproteins) following a vitamin A fat-loading test, 36h after the final session of 7 weeks of jogging. Together, such findings suggest that the postprandial influence of MICT declines between 36 and 48h post-exercise.

Detraining studies suggest that heightened control of PPL in those who are habitually trained, appears only to be a transient result of recent training bouts (Gill & Hardman, 2003). Indeed, 60h post-exercise, postprandial TAG AUC was already 35% greater than at 15h post-exercise in endurance trained subjects (Hardman, Lawrence, & Herd, 1998). Similarly, after just 60h of detraining, following 13 weeks of running, both males and females exhibited a 37% increase in postprandial TAG AUC, rising to 46% after 9 days (Herd, Hardman, Boobis, & Cairns, 1998).

Nevertheless, the possibility of training-induced adaptations should not be entirely ruled out. Despite trained and untrained women exhibiting no postprandial differences when assessed without prior exercise, trained women demonstrated greater reductions in postprandial TAG 16h after acute exercise (Tsetsonis, Hardman, & Mastana, 1997). The authors suggested that the lower insulin response in trained women may denote decreased adipose, and increased skeletal muscle LPL activity and a subsequently heightened uptake of TAG into muscle.

### 1.7.2 High Intensity Interval Training

Investigating the effects of chronic HIIT on PPL represents a marriage between the growing support for HIIT as a time-effective means to improve health, and the clinical relevance of assessing non-fasting TAG to evaluate CVD risk. Owing to the relative novelty of the field, literature which measures postprandial outcomes ≥72h after the final training bout, to disregard transient effects, is currently scarce.

Given the wealth of research suggesting chronic MICT confers little enduring benefits to lipaemia, it would be easy to assume that the same would hold true for HIIT. It must be recalled, however, that while the acute

responses to HIIT were similar in magnitude to those of MICT, mechanistically they appeared to differ. Unlike MICT, HIIT does not require ≥2MJ energy expenditure to elicit an acute response, and appears to decrease hepatic VLDL-TAG secretion without affecting clearance (Maraki & Sidossis, 2013). Additionally, despite similar acute activation of signalling cascades associated with mitochondrial biogenesis following work-matched HIIT and continuous training, 6 weeks of the latter did not alter mitochondrial enzyme activities, which contrasts those previously observed using the HIIT protocol (Cochran et al., 2014). It is possible, therefore, that HIIT may confer different long-term outcomes, arising from different signalling pathways and resultant adaptations. It is likely that any such outcomes would be most prominent in those who typically exhibit greatest lipaemia-lowering responses to acute exercise such as the sedentary and obese.

Following an acute bout of HIIT, Freese (2015) found a significant reduction in postprandial TAG before and after 6 weeks of regular training in women at risk of the metabolic syndrome. These reductions were not different between time points, however, suggesting no accumulative effect of training. It is important to note that the study utilised an abbreviated 3h postprandial assessment. While 3h assessments may be predictive of 6h outcomes in healthy individuals ($R^2$ = 0.43 - 0.92), in those who present with hyper-TAG, (a marker of the metabolic syndrome risk), the relationship is not significant (Maraki, Aggelopoulou, Christodoulou, Katsarou, et al., 2011). The results reported by Freese, therefore, may not represent full postprandial TAG responses to HIIT in the sampled population. The study utilised 4x30s all-out sprints on a cycle ergometer. The requirement of a specialist ergometer and to repeatedly work at maximal effort limits such interventions in their capacity to be replicated, unsupervised, outside of the laboratory. Thus, such protocols are not reflective of realistic lifestyle interventions that might be employed by the general population.

Employing the more contemporary 10x60s HIIT model, (Bond, Cockcroft, et al., 2015) studied the effects of 6 sessions over 2 weeks in healthy

adolescents. No changes were found in fasting, AUC or iAUC TAG, glucose, or insulin at either 1 or 3-days post training, when assessed for 4h after feeding. In contrast, both FMD of the brachial artery and heart rate variability in the fasted state were significantly improved at both time points, suggesting reduced CVD risk, despite the absence of improvements in more conventional measures. Given the young age (13-14 years) and good health of the participants, they likely presented very low levels of atherosclerotic lesions. It is possible that this resulted in smaller magnitude changes, and/or that the 2-week duration was insufficient to reap detectable benefits in traditional CVD risk markers. Recent findings suggest this to be a plausible hypothesis. Four days after the final session of 10 weeks of regular sports-based HIIT (4-7x45s maximal effort drills, eliciting ~90% $HR_{max}$) non-fasting TAG was 26% lower in a training versus control group, in a study of 101 healthy 14 year olds (Weston et al., 2016). It should be noted, however, that while factored into analysis as a covariate, the duration since the last feeding was neither standardised nor reported.

At present, no study has rigorously assessed the existence of non-transient effects of HIIT training on postprandial lipaemia and further metabolic markers, in healthy adults. While existing research speculates that such effects may be small or even non-existent, it is important to investigate such possibilities, as positive effects could have significant public health implications regarding HIIT within physical activity guidelines.

## 1.8 Aims

There exists a dearth of chronic HIIT studies assessing postprandial lipaemia, especially those which examine non-transient effects. Hence, this thesis seeks to contribute to the existing literature by investigating the chronic effects of HIIT, which could be replicated outside of the laboratory, on postprandial lipaemia, in healthy males.

To enhance mechanistic understanding of the processes responsible for any such findings, or the absence thereof, additional measures of traditional and contemporary CVD risk factors will be concomitantly assessed before and after the intervention. Such results will help direct future research by providing insight into the role of intensity and duration in the protective effects of HIIT. Furthermore, findings may help to inform future policy with regards to the inclusion or exclusion of HIIT within physical activity guidelines.

# Chapter 2:

# Methods

## 2.1 Study design

This is a repeated measures study, involving invasive (blood and muscle tissue samples) and non-invasive (cardiopulmonary exercise testing, indirect calorimetry, blood flow, urine samples) assessment of metabolic, vascular, and cardiopulmonary function, pre- and post-four-week HIIT intervention in healthy, young males.

## 2.2 General Proceedings

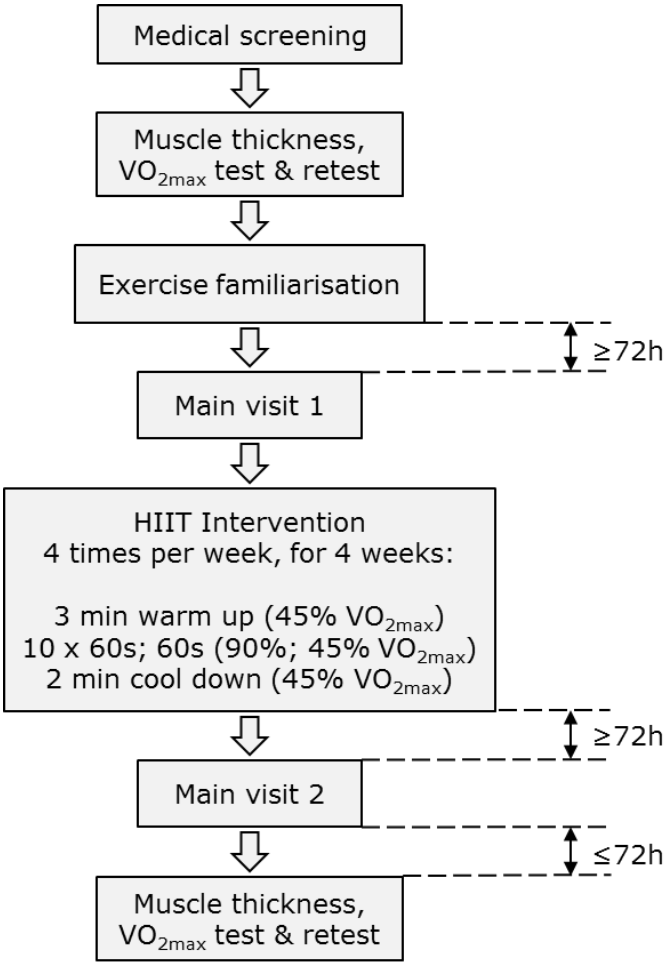


**Figure 3.** Schematic diagram of the structure of the study.

## 2.3 Participants

Eight male participants (22 ± 3 years, 1.77 ± 0.07m, 67.7 ± 6.2kg) were recruited following advertisement on university notice boards and social media platforms. Participants, comprising students from the University of Nottingham, were invited to participate voluntarily. Following written informed consent, participants underwent a routine health screening, including anthropometric measures, blood pressure, blood samples, an electrocardiogram and an International Physical Activity Questionnaire (IPAQ). Eligibility was defined as BMI <28kg·m$^{-2}$; non-smoker; no known health conditions; not taking prescription medication; and an IPAQ classification below 'health enhancing'. Participants reported spending an estimated 46.4 ± 9.3, 6.3 ± 3.3, 1.3 ± 1.6 and 0.7 ± 0.8h·week$^{-1}$ sitting, walking, moderately and vigorously exercising, respectively. Participants were instructed to maintain habitual physical activity throughout the study.

## 2.4 Cardiopulmonary Exercise Testing

Participants undertook an incremental cardiopulmonary exercise test (CPET) on an electronically braked cycle ergometer (Excalibur, Lode B.V., Groningen, NL) to determine maximal oxygen uptake ($VO_{2max}$) using breath-by-breath analysis (Quark, Cosmed, Rome, IT), and corresponding heart rate (RS400, Polar Electro Oy, FI). The test commenced at 1W·kg$^{-1}$, at 70RPM. Power was increased 20-40W every 3min, until near-maximal effort was reached (respiratory exchange ratio (RER) approaching 1.1 and near-maximal rating of perceived exhaustion), where after, smaller increments were made until volitional failure or a clear plateau/decline in $VO_2$ and an RER>1.10 was achieved. The mean $VO_2$ of the final 30s (Mezzani, 2017) of the minute of highest mean $VO_2$ was considered $VO_{2max}$. After >30min of rest, to confirm $VO_{2max}$, a second, abbreviated test utilising larger increments (40-80W) was performed until a workload ~20W greater than the final stage of the first test was achieved. The workload and heart rate corresponding to 90% and 45% $VO_{2max}$, were subsequently determined for training intensities.

## 2.5 Dietary and Physical Activity Standardization

In advance of the study, participants completed a 3-day self-reported diet diary for energy intake and macronutrient assessment (Nutritics Professional, IE). Following dietary suggestions aiming to produce similar macronutrient intake between participants (45% CHO, 35% fat 20% protein), a second diary was completed for 3 days prior to the first main study visit. During this period, participants were asked to abstain from alcohol and to avoid physical activity beyond that required for normal living. The last meal before the visit was standardised, providing 561kcal comprising 41g CHO, 27g fat and 33g protein. Participants fasted overnight for ≥12h with only water. For the second main visit, participants recorded a third 3-day diary, replicating the second as closely as possible. Transport to the laboratory was provided for main visits.

## 2.6 Main Visit Proceedings

Prior to and ≥72h following the first and last HIIT sessions, participants attended the laboratory to provide a sample of muscle tissue and to undergo a 6h mixed meal tolerance test (Fig 4). Upon arrival participants provided a urine sample, before their body mass was measured.

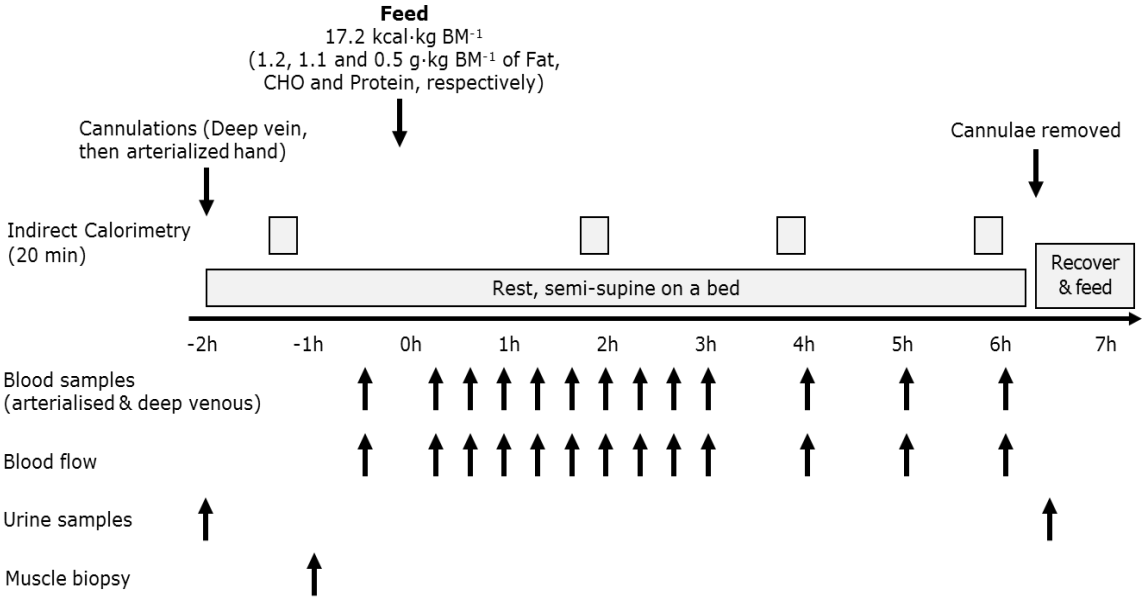


**Figure 4.** Schematic diagram of the main study visit, which was undertaken pre- and post-training intervention.

### 2.6.1 Bergstrom Muscle Biopsy

While fasted, a sample of muscle tissue was extracted from the thigh using the Bergstrom biopsy technique. Participants rested with the lower limbs relaxed. The *vastus lateralis* (a knee extensor muscle heavily implicated in cycling) was cleaned with an iodine solution before the administration of ~7mL of 1% lignocaine. A small incision was made, into which the Bergstrom needle (Fig. 5) was inserted (Shanely et al., 2014). Suction was applied to the inner trocar via a syringe, and the outer trocar retracted, drawing muscle tissue into the opening. Rapid closing of the outer trocar cut a small sample of tissue, which was repeated several times. Tissue was snap frozen in liquid nitrogen-cooled isopentane. Sustained pressure was applied to the site of incision, before dressing the wound. While the procedure does not limit normal daily functions, participants were advised to avoid vigorous physical activity for 72h. Samples were stored in liquid nitrogen until analysis.


**Figure 5.** Bergstrom biopsy needle comprising an outer cannula with a small opening near the tip (A), an inner trocar with a cutting blade (B) and a pushing rod to expel collected tissue (C).

### 2.6.2 Tolerance Test Meal

A standardised meal of croissants, butter, jam and eggs, with a whole milk, double cream, sugar and cocoa powder drink was consumed. The meal was prescribed according to the participant's fasted body mass, providing 17.6kcal·kg$^{-1}$, comprising 1.2g·kg$^{-1}$ fat (of which 60% saturated, and 25% and 7% mono- and polyunsaturated, respectively), 1.2g·kg$^{-1}$ CHO (of which 54% sugars) and 0.5g·kg$^{-1}$ protein, accompanied by 2mL·kg$^{-1}$ water. Consumption of the meal was paced to last 10 min, the end of which was considered time = 0min post-meal.

### 2.6.3 Blood Sampling

Two retrograde cannulas were employed. One in a superficial vein of one hand which was kept in a hot box at 55°C to 'arterialise' the blood, the other guided into a deep vein of the opposite arm using ultrasound. Blood samples were drawn from both lines simultaneously to assess arterio-venous differences. Samples of 7mL were collected immediately before and every 20min after the consumption of the test meal for 3h, then hourly for a further 3h. For the assessment of blood glucose, 0.5mL of whole blood was rolled in fluoride-containing microtubes for 3min before analysis (YSI 2300, Yellow Springs Instruments, OH, US). Four millilitres of blood were aliquoted into a tube containing 30μL EGTA-glutathione, and 2.5mL into a spray-coated silica and polymer gel tube, the latter being allowed to clot, before 10min centrifugation at 4400g at 4°C to separate plasma and serum, respectively. Plasma was aliquoted into Eppendorfs containing 5μL of tetrahydrolipstatin for the assessment of free fatty acids. Serum was aliquoted for the assessment of triglycerides, insulin and branched chain amino acids. Samples were stored at -80°C until analysis.

### 2.6.4 Blood Flow and Substrate Uptake

Images and videos of the brachial artery of the non-arterialised arm were obtained immediately after each blood sample was collected. With the participant laying on a gently inclined bed with the arm supported, the brachial artery was visualised longitudinally, 2-5cm proximal to the antecubital fossa via B-mode ultrasonography with a 12MHz linear array transducer (Aplio 300, Toshiba, JP). Once a clear image was obtained, the transducer location was marked to enable accurate repeated placement. Three measures of brachial artery diameter (BAD); the distance from the anterior to posterior 'm' line (the interface between the media and adventitia), were taken at end systole ($D_s$) and end diastole ($D_d$), from a simultaneous electrocardiogram (Fig. 6; A). Flow velocity was measured using a pulsed doppler signal at 60° to the artery with a ~2mm range gate (Fig. 6; B). All measurements were performed by the same investigator.

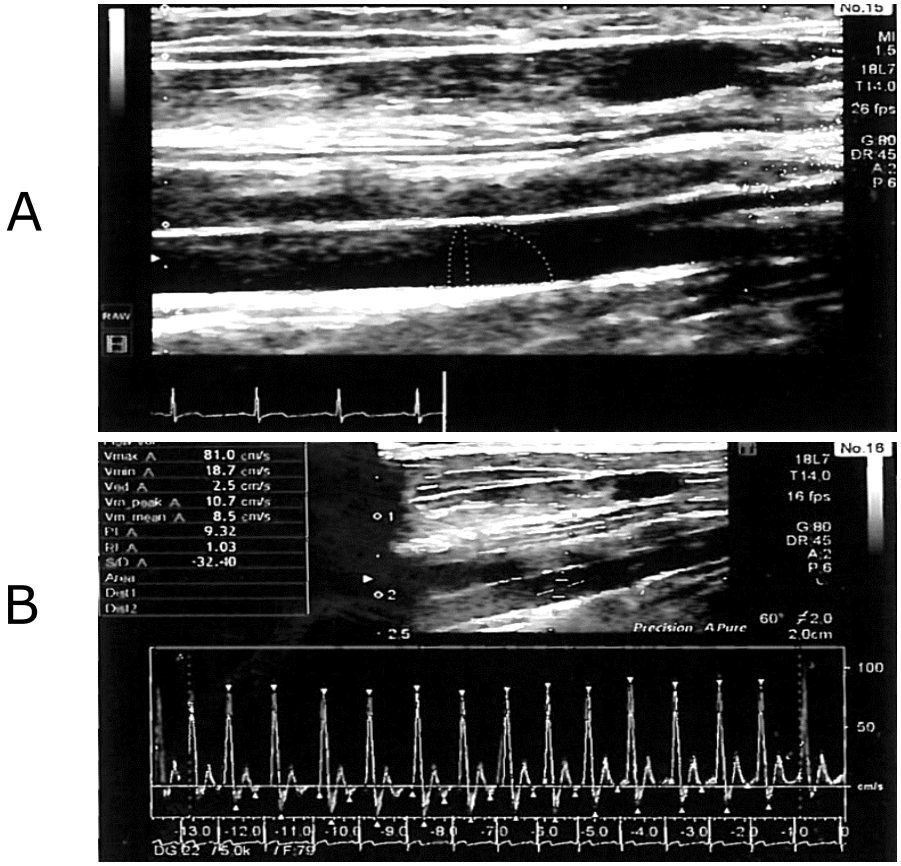


**Figure 6.** Ultrasound image from the determination of brachial artery diameter (A) and flow velocity (B) in a representative subject.

Forearm blood flow ($BF_f$; $cm^3 \cdot min^{-1}$ alternatively $mL \cdot min^{-1}$) was derived from BAD and its corresponding flow velocity ($v$; $cm \cdot s^{-1}$):

$$BF_f = (BAD \times v) \times 60$$

Time-matched substrate concentration values (glucose, TAG, FFA and BCAA) were factored in to determine uptake, extraction%, and clearance across the forearm:

$$Substrate\ Uptake = (A_{sub} - V_{sub}) \times BF_f$$

$$Substrate\ Extraction = \left(\frac{A_{sub} - V_{sub}}{A_{sub}}\right) \times 100$$

$$Substrate\ Clearance = \left(\frac{A_{sub} - V_{sub}}{A_{sub}}\right) \times BF_f$$

Where $A_{sub}$ and $V_{sub}$ are the substrate concentrations (glucose, TAG and FFA in $mmol \cdot L^{-1}$ and BCAA in $\mu mol \cdot L^{-1}$) at any given time point, of arterialised and venous blood, respectively.

### 2.6.5 Muscle Thickness

Muscle Thickness (MT) of the *vastus lateralis* (VL), *vastus intermedius* (VI), and *rectus femoris* (RF) was assessed by the same investigator from images obtained *in vivo,* at rest, using B-mode ultrasonography (Aplio 300, Toshiba, JP), with a 40mm, 12MHz linear-array probe. The medial and lateral borders of the VL were marked on the leg and resting images were taken at 50% of femur length, measured from the central point of the patella to the medial aspect of the superior iliac spine. The transducer was placed longitudinally to the thigh and three images of each muscle were acquired and stored for offline analysis. MT was identified as the distance between superficial and deep aponeuroses, taken from the distal portion of the acquired image (Fig. 7) using the ImageJ 1.42q software (National Institutes of Health, US).

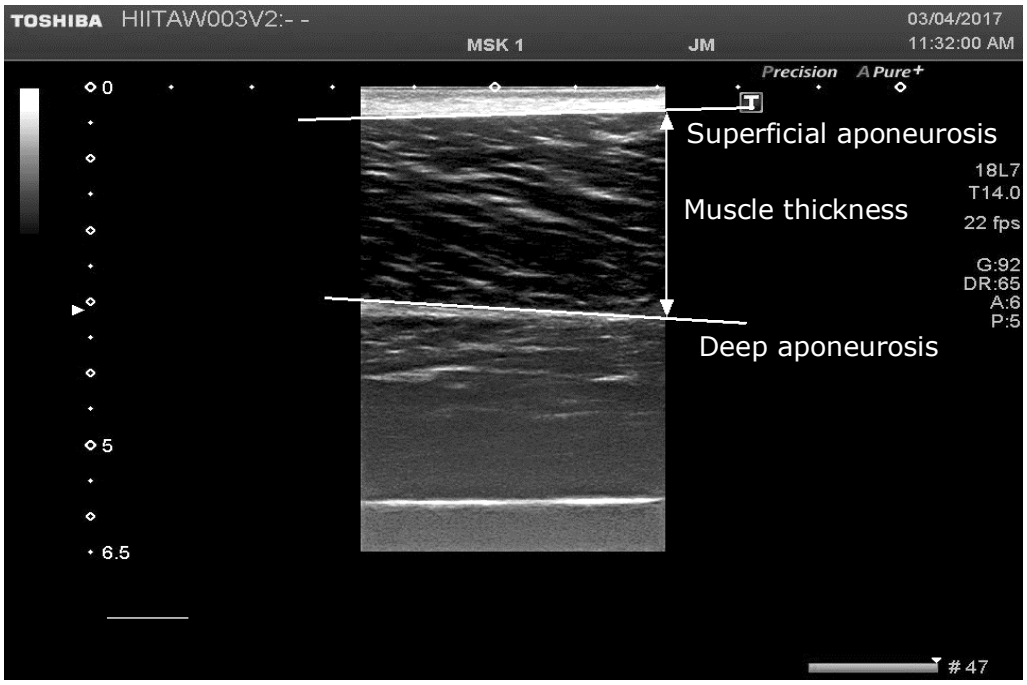


**Figure 7.** Ultrasound image of the vastus lateralis muscle (at 50% of femur length) in a representative subject with muscle thickness measurement highlighted (solid line between aponeuroses).

### 2.6.6 Indirect Calorimetry

Resting substrate oxidation and energy expenditure was assessed using indirect calorimetry pre, and 2, 4 and 6h post feeding. Expired gas was sampled in 10s bins for 20min using a flow-based dilution canopy hood (Quark RMR, Cosmed, IT), the first 5min of which was discarded. Urine samples were collected, and their volumes measured, before, during and after the tolerance test and stored at -80°C until analysis.

Urea concentrations were quantified in urine and plasma (from the first and last blood samples) using a commercially available enzymatic kinetic assay (UR220, Randox, NI). Briefly, in the presence of water, urea is hydrolysed to urease, producing ammonia and $CO_2$. Ammonia combines with α-oxoglutarate and NADH in the presence of glutamate-dehydrogenase, to yield glutamate and NAD+. Following addition of the reagent, absorbance was read at 340nm (SpectraMAX 190, Molecular Devices LLC, CA, US) after 30s and 30min of incubation at 37°C. Urea

concentrations were calculated from the regression equation of a set of known standards. Nitrogen excretion rates were then estimated:

$$Nitrogen\ Excretion = \left(\frac{Urea_{urine} \times Vol_{urine} \times 0.47}{t_2 - t_1}\right) + \left(\frac{\Delta\ Urea_{plasma} \times 0.47 \times 0.57}{t_2 - t_1}\right)$$

Where nitrogen excretion ($g \cdot min^{-1}$) is determined from urea concentrations ($g \cdot mL^{-1}$), urine volume (mL), and where $t_2 - t_1$ is the time between the first and last samples (min). The following assumptions were made: that 47% of urea is nitrogen; that 57% of body mass is water; and that during the 12h fast, plasma urea was unchanged.

Nitrogen excretion rates were used to correct indirect calorimetry data using the equations from Frayn (1983):

$$VO_2\ (l \cdot min^{-1}) = (0.746 \times CHO) + (2.03 \times F) + (6.04 \times n)$$

$$VCO_2\ (l \cdot min^{-1}) = (0.746 \times CHO) + (1.43 \times F) + (4.89 \times\ n)$$

Where the subject is oxidizing CHO and F grams of carbohydrate and fat per minute, respectively, while excreting n grams of nitrogen per minute. Hence, assuming negligible lipogenesis, gluconeogenesis and ketogenesis, for any given rates of $VO_2$, $VCO_2$, nitrogen excretion and substrate oxidation rates ($g \cdot min^{-1}$) can be determined as follows:

$$CHO\ oxidation = (4.55 \times VCO_2) - (3.21 \times VO_2) - (2.87 \times n)$$

$$Fat\ oxidation = (1.67 \times VCO_2) - (1.67 \times VO_2) - (1.92 \times n)$$

Since postprandial urine was pooled, and plasma urea determined from samples at t=0 and t=6h, calculated nitrogen excretion rates will not perfectly represent all time points. To adjust for this, if Frayn's equations yielded negative values of CHO or fat oxidation, non-corrected indirect calorimetry data was substituted (4 out of 128 values substituted).

Resting metabolic rate (RMR; expressed as $kcal \cdot min^{-1}$) was determined from uncorrected indirect calorimetry data using the Weir equation (Weir, 1949):

$$RMR = (3.941 \times VO_2) + (1.1106 \times VCO_2)$$

## 2.7 HIIT Protocol

Training sessions utilised an electronically braked cycle ergometer (Corival, Lode B.V., NL). Prior to commencing the study, participants completed one HIIT session for familiarisation. Training began with a 3min warm-up at a power corresponding to 45% $VO_{2max}$. Participants then performed 10x60s bouts at 90% $VO_{2max}$, separated by 60s bouts at 45% $VO_{2max}$. Participants cycled for a further 2mins at 45% $VO_{2max}$ to cool down. Throughout the intervention, heart rate was regularly monitored and workloads increased accordingly to best attain a rate corresponding to 90% $VO_{2max}$.

## 2.8 Sample Analysis

### 2.8.1 Muscle Protein Extraction and Quantification

For the extraction of whole cell muscle protein lysate, ~30mg of wet tissue was first homogenised with a polytron in 300µl HEPES buffer. After 20min resting on ice, the homogenate was centrifuged at 10,000g for 20min at 4°C and the supernatant transferred to a clean Eppendorf. Total protein concentration was determined using the Pierce bicinchoninic acid (BCA) assay (Thermo Fisher Scientific, MA, US). Briefly, Samples were diluted to 1 in 10 in distilled water. Ten microliters of stock or protein standard, and 200µl of reagent were pipetted into the wells of a microplate and incubated at 37°C for 30min. Absorbance was read at 562nm on a spectrophotometer (SpectraMAX 190). Protein concentration of each sample was subsequently calculated using the regression equation from the known protein standards.

### 2.8.2 Western Blotting

Protein concentration of individual stocks were standardised to 2.5µg·µL$^{-1}$ with sodium dodecyl sulphate (SDS) loading buffer (containing bromophenol blue) and a diluent of HEPES buffer and a protease inhibitor cocktail, before boiling at 95°C for 5 min. Fifty micrograms of total muscle lysate proteins were loaded into the wells of a 12% SDS polyacrylamide gel for separation via electrophoresis (SDS-PAGE) in a vertical, dual plate

tank. Following electrophoresis, the gel-bound separated proteins were electroblotted overnight to polyvinylidene difluoride membranes and subsequently blocked in 5% (w/v) Marvel milk or bovine serum albumin (BSA) in tris buffered saline containing 0.1% (w/v) tween 20 (TBS-T) for 1h at room temperature.

Membranes were incubated overnight at 4°C with primary antibodies for LPL (mouse, ab21356, abcam, UK; 1 in 1000 concentration in 5% (w/v) milk TBS-T), and adipose triglyceride lipase (ATGL) (rabbit, ab1092951, abcam; 1 in 1000 in 5% (w/v) BSA TBS-T). Membranes were also probed for anti-αactin (rabbit, A2066, Sigma-Aldrich, MO, USA; 1 in 5000 in 1% (w/v) milk TBS-T) and cyclophilin B (CypB) (mouse, ab74173, abcam; 1 in 4000 in 5% (w/v) BSA TBS-T) to serve as an endogenous control. After 5x5min TBS-T washes, membranes were incubated for 1h at room temperature with a horse radish peroxidase-linked secondary antibody (goat anti-mouse, Dako, DK: 1 in 2000 in 5% (w/v) milk TBS-T; swine anti-rabbit, Dako: 1 in 2000 in 5% (w/v) BSA TBS-T; swine anti-rabbit, Dako: 1 in 2000 in 1% (w/v) milk TBS-T; and goat anti-mouse, Dako; 1 in 2000 in 3% (w/v) BSA TBS-T, for the detection of primary antibodies for LPL, ATGL, anti-αactin, and CypB, respectively). After 3x10min washes, proteins were visualised using an enhanced chemiluminescence detection reagent and X-ray film (ECL prime and Hyperfilm, Amersham Biosciences, UK). The resulting bands were quantified using densitometry software (Aida Image Analyser v.4.27, Raytest Isotopenmessgeräte, DE) and proteins of interest were normalised against the endogenous controls.

### 2.8.3 Circulating Triglyceride

Serum TAG concentrations were determined by coupled enzymatic colorimetry using a clinical chemistry analyser (ABX Pentra 400, Horiba Ltd., JP). Triglycerides within serum are hydrolysed by LPL to give glycerol and fatty acids. Adenosine triphosphate phosphorylates glycerol in the presence of glycerokinase to form glycerol-3-phosphate (G3P) and adenosine diphosphate. The G3P is oxidised by molecular oxygen via the action of G3P-oxidase, giving hydrogen peroxide ($H_2O_2$) and

dihydroxyacetone phosphate. The $H_2O_2$ reacts with p-Chlorophenol and 4-aminoantipyrie in the presence of peroxidase, forming a quinonimine chromophore. Increased light absorbance from the resulting chromophore is proportional to sample TAG concentration (Bucolo & David, 1973; Trinder, 1969).

### 2.8.4 Circulating Free Fatty Acids

Plasma free fatty acid concentrations were determined via coupled enzymatic colorimetry (ABX Pentra 400) using a commercially available kit (NEFA HR-2, Wako, JPN). Briefly, fatty acid acylation of coenzyme A (CoA) is catalysed by the action of acyl CoA synthetase. The resulting acyl-CoA is oxidised by acyl-CoA Oxidase to produce $H_2O_2$ which reacts with 4-Aminoantipyrine and a colorimetric probe to produce a quinonimine chromophore (Mulder, Schouten, & Popp-Snijders, 1983). Subsequent change in absorbance at 550nm is proportional to sample FFA concentration.

### 2.8.5 Circulating Insulin

Serum insulin concentrations were determined using a commercially available radioimmunoassay (HI014K, Merk Millipore, MA, US) via the double antibody/polyethylene glycol technique (Heding, 1972). A fixed concentration of $^{125}$I-labelled Human Insulin is incubated with a constant dilution of antiserum to limit antigen binding sites to ~50%, creating competition when unlabelled antigen (from serum) is added. As unlabelled antigen concentration increases, antibody-bound tracer is competitively displaced and its concentration decreases. Bound antigens are separated from their unbound counterparts and the radioactivity of the remaining unbound antigen is measured with a gamma counter. Sample insulin concentration is proportional to the radioactivity of a set of known concentrations of unlabelled antigen.

Homeostasis model assessments of insulin resistance (HOMA2-IR) was carried out using HOMA calculator version 2.2.2 (http://www.dtu.ox.ac.uk/ accessed August 2017).

Matsuda insulin sensitivity index values were calculated as:

$$Matsuda\ Index = \frac{10000}{\sqrt{(Glu_0 \times Ins_0 \times Glu_{mean} \times Ins_{mean})}}$$

Where $Glu_0$ and $Ins_0$ are the fasting concentrations of glucose and insulin, while in accordance with previous research $Glu_{mean}$ (mmol·L$^{-1}$) and $Ins_{mean}$ (ρmol·L$^{-1}$) are their mean concentrations for the first 120min of postprandial measurement (Aloulou, Brun, & Mercier, 2006; Kevin C. Maki, McKenney, Farmer, Reeves, & Dicklin, 2009).

### 2.8.6 Branched Chain Amino Acids

Total serum concentration of branched-chain amino acids (BCAA) was determined spectrophotometrically. Briefly, leucine, isoleucine and valine are oxidatively deaminated by leucine dehydrogenase with stoichiometric reduction of nicotinamide adenine dinucleotide (NAD) (Ohshima, Misono, & Soda, 1978). Five microliters of serum (measured singularly) or standard (in duplicate) were loaded with 285μL glycine- potassium chloride- potassium hydroxide buffer, containing NAD. Changes in absorbance at 340nm, corresponding to NADH formation (reduced NAD; proportional to BCAA concentration) were measured before and 60min after the addition of leucine dehydrogenase within a sodium phosphate buffer. Outlying or missing values (a consequence of measuring samples singularly), were imputed as the mean of concentrations 1h prior to and post the value in question (10 out of 224 values imputed).

## 2.9 Statistical Analysis

Descriptive statistics are presented as mean ± standard deviation (SD). All subsequent data are presented as mean ± standard error of the mean (SEM). Tests of normality were performed in SPSS 22.0 using Shapiro-Wilk tests (IBM, NY, US). If data were acceptably normal or only a few time points exhibited small to moderate departures from normality, two-way repeated measures analysis of variance (ANOVA) were employed to detect differences between pre-and post-HIIT data (training effect), across

multiple time points (time effect) in Prism 7 (GraphPad Software Inc., CA, USA). Statistical significance was defined as $P<0.05$. If ANOVA revealed a significant main effect, post-hoc Bonferroni corrected multiple comparisons tests were performed for pre- and post-HIIT comparisons between time-points. To differentiate between fasting and postprandial changes, both total and incremental AUCs were calculated using the trapezoid rule, with iAUC determined by first subtracting the fasting concentration from all postprandial concentrations (Freese et al., 2015). Group means were compared using paired samples t-tests. Pearson product-moment correlation was used to assess the relationship between baseline $VO_{2max}$ and its magnitude of change following training.

# Chapter 3:

# Results

## 3.1 Characteristics, Completion and Standardisation

Participants completed all 16 HIIT sessions in 24 ± 2 days (mean ± SD), with no adverse outcomes. Post-training main visits and cardiopulmonary exercise tests were carried out 78 ± 10h and 114 ± 24h after the final training session, respectively. Participant characteristics are described in Table 1.

**Table 1.** Participant physical characteristics before (baseline) and after 16 sessions of HIIT (post).

| | Baseline | Post | *P*-value |
|---|---|---|---|
| Age (y) | 22.1 (± 3.2) | - | - |
| Height (m) | 1.77 (± 0.07) | - | - |
| Body Mass (kg) | 67.66 (± 6.22) | 67.86 (± 6.31) | 0.57 |
| Body Mass Index ($kg \cdot m^{-2}$) | 21.61 (± 1.88) | 21.67 (± 1.83) | 0.60 |
| $VO_{2max}$ ($mL\ O_2 \cdot kg^{-1} \cdot min^{-1}$) | 47.30 (± 5.46) | 51.80 (± 3.41) | 0.004 |

Data presented as mean (± SD); n=8.

Energy intake was not different between the pre- and post-training standardised period (2145 ± 93 vs. 2092 ± 126kcal, *P* = 0.41) (Fig. 8). Energy contribution from CHO, fat and protein was also not different between pre- and post-training (46.3 ± 2.3 vs. 45.1 ± 3.4% CHO, *P* = 0.58; 35.1 ± 2.0 vs. 36.3 ± 3.2% fat, *P* = 0.62, and 18.6 ± 0.8 vs. 18.6 ± 1.1% protein, *P* = 0.97).

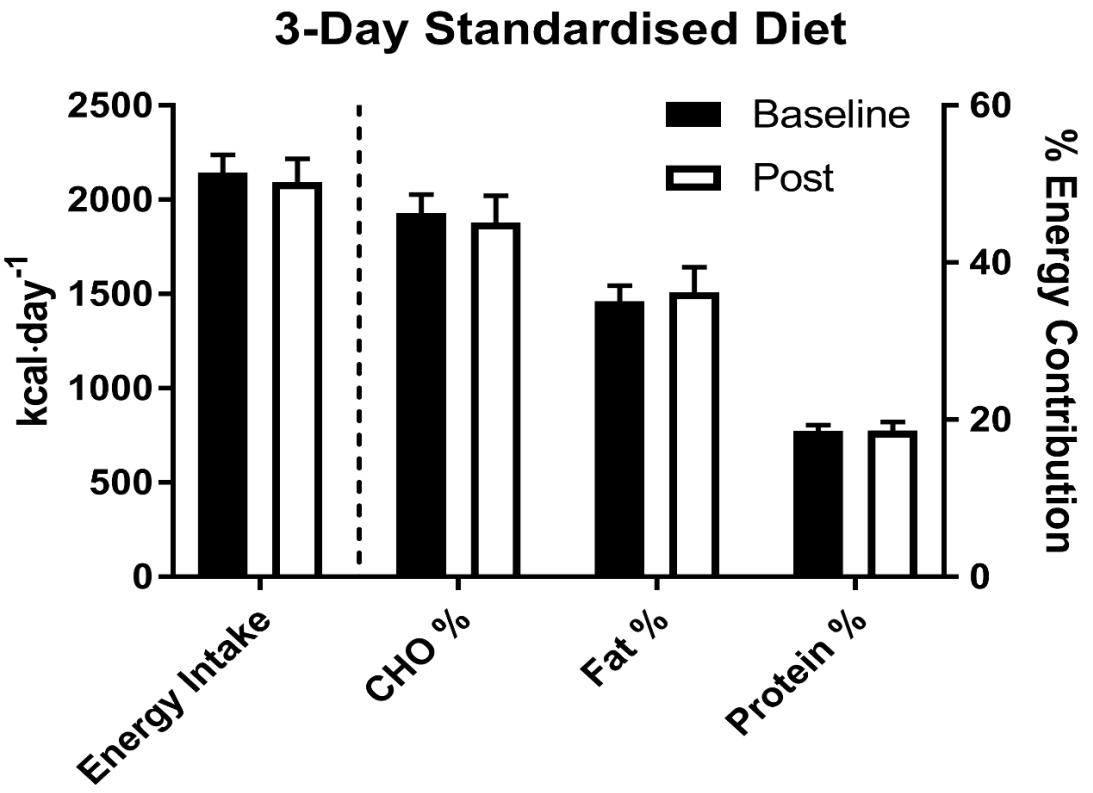


**Figure 8.** Three-day energy intake and macronutrient contribution prior to the baseline and post-intervention main visits. Data determined from self-reported diet diaries using Nutritics dietary analysis software. Data displayed as mean ± SEM; n=8.

## 3.2 Cardiopulmonary and Musculoskeletal Responses

Maximal oxygen uptake increased by 10.2 ± 7.7% with training ($P$ = 0.004) (Fig. 9; A). Pearson's product moment revealed a strong negative correlation (r = -0.88, $P$ = 0.004) between baseline $VO_{2max}$ and $\Delta\%VO_{2max}$ (Fig. 9; B). Across the 16 sessions of HIIT, workload corresponding to 90% $VO_{2max}$ was increased by 22 ± 9W (11 ± 4%).

Due to time limitations, quadriceps muscle thickness (MT) was only measured in four participants. Because the protocol used fixed-load cycle ergometry, it is reasonable to speculate that any hypertrophic response would occur equally in each leg. Thus, both right and left leg MT data were pooled to increase statistical power. Thickness of the *vastus lateralis* and *rectus femoris* was significantly increased after HIIT (2.31 ± 0.07 vs. 2.59 ± 0.10cm, $P$ = 0.02, and 1.77 ± 0.08 vs. 1.89 ± 0.09cm, $P$ = 0.01, respectively) (Fig. 10). Neither medial nor lateral *vastus intermedius* MT was significantly changed (1.61 ± 0.09 vs. 1.53 ± 0.05, $P$ = 0.08, and 2.43 ± 0.07 vs. 2.42 ± 0.09, $P$ = 0.96, respectively).

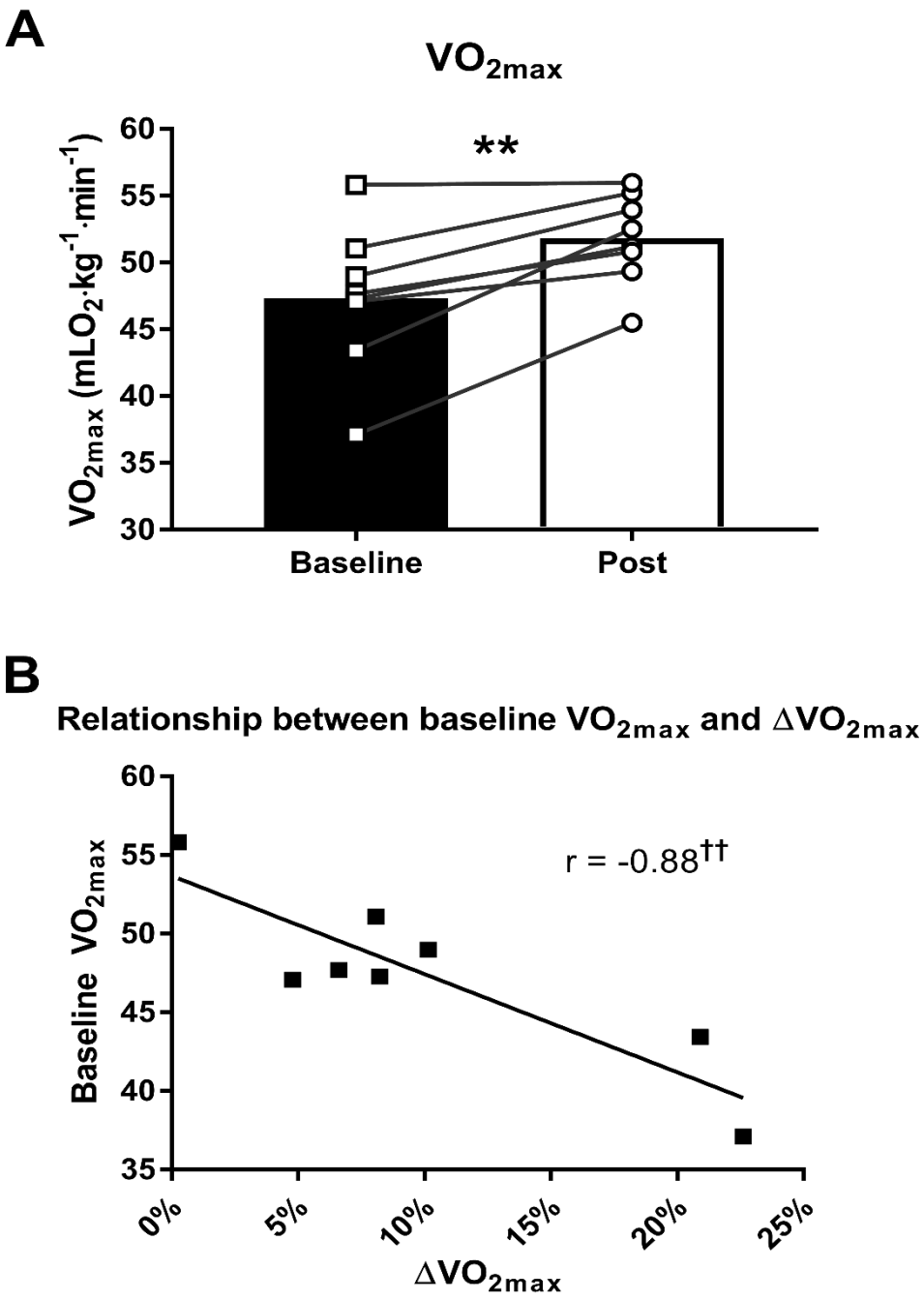


**Figure 9.** Mean (vertical bars) and individual (horizontal lines) changes in maximal oxygen uptake ($VO_{2max}$) before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8 (A). The relationship between baseline $VO_{2max}$ and percent change $VO_{2max}$, quantified using Pearson's product moment, after 16 sessions of HIIT; n=8 (B). Maximal oxygen uptake significantly increased with training, $^{**}P < 0.01$. Baseline $VO_{2max}$ negatively correlated with $\Delta VO_{2max}$, $^{\dagger\dagger}P < 0.01$.

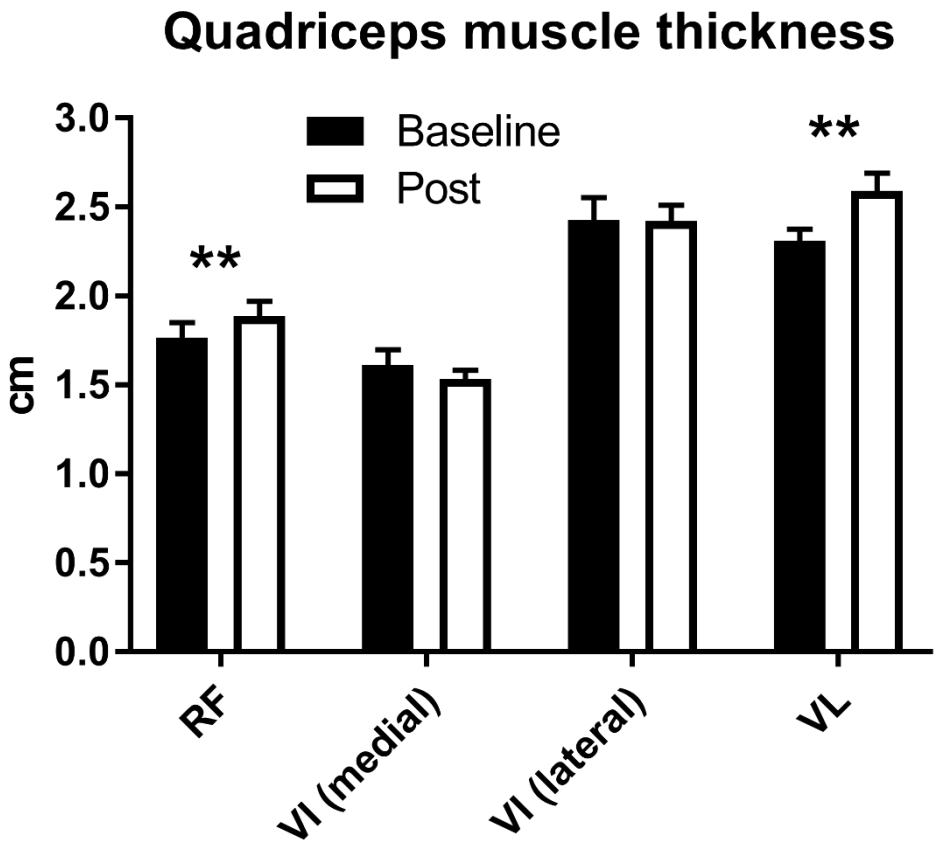


**Figure 10.** Changes in muscle thickness of the *rectus femoris* (RF), the medial and lateral portions of the *vastus intermedius* (VI), and *vastus lateralis* (VL), of both the right and left leg, measured at 50% of femur length via B-mode ultrasonography, before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=4. Muscle thickness of the RD and VL significantly increased with training, $^{**}P < 0.01$.

## 3.3 Resting Substrate Oxidation and Energy Expenditure

When protein-oxidation-corrected indirect calorimetry data was analysed, paired two-tailed t-tests found no change in fasting CHO or fat metabolism after four weeks of HIIT (0.14 ± 0.04 vs. 0.13 ± 0.03g·min$^{-1}$, $P$ = 0.87, and 0.07 ± 0.02 vs. 0.08 ± 0.02g·min$^{-1}$, $P$ = 0.75, respectively). Repeated measures ANOVA revealed no main effect of HIIT on oxidation rates of CHO ($P$ = 0.41), a significant effect of time ($P$ = 0.02), but no time × condition interaction ($P$ = 0.34) (Fig. 11; A). No main effects of training or time, and hence no interaction, were observed in fat oxidation rates ($P$ = 0.18, $P$ = 0.19, and $P$ = 0.26, respectively) (Fig. 11; B). ANOVA revealed no main effect of training, nor time on subsequent whole body RER ($P$ = 0.38 and $P$ = 0.38, respectively), and hence no interaction between the two ($P$ = 0.33). Fasting RMR was not influenced by training (1839 ± 82 vs. 1870 ± 52kcal·day$^{-1}$, $P$ = 0.55). A significant effect of time ($P$ < 0.01), but not training ($P$ = 0.24) was found on RMR, and no interaction effect was detected ($P$ = 0.58) (Fig. 11; D).

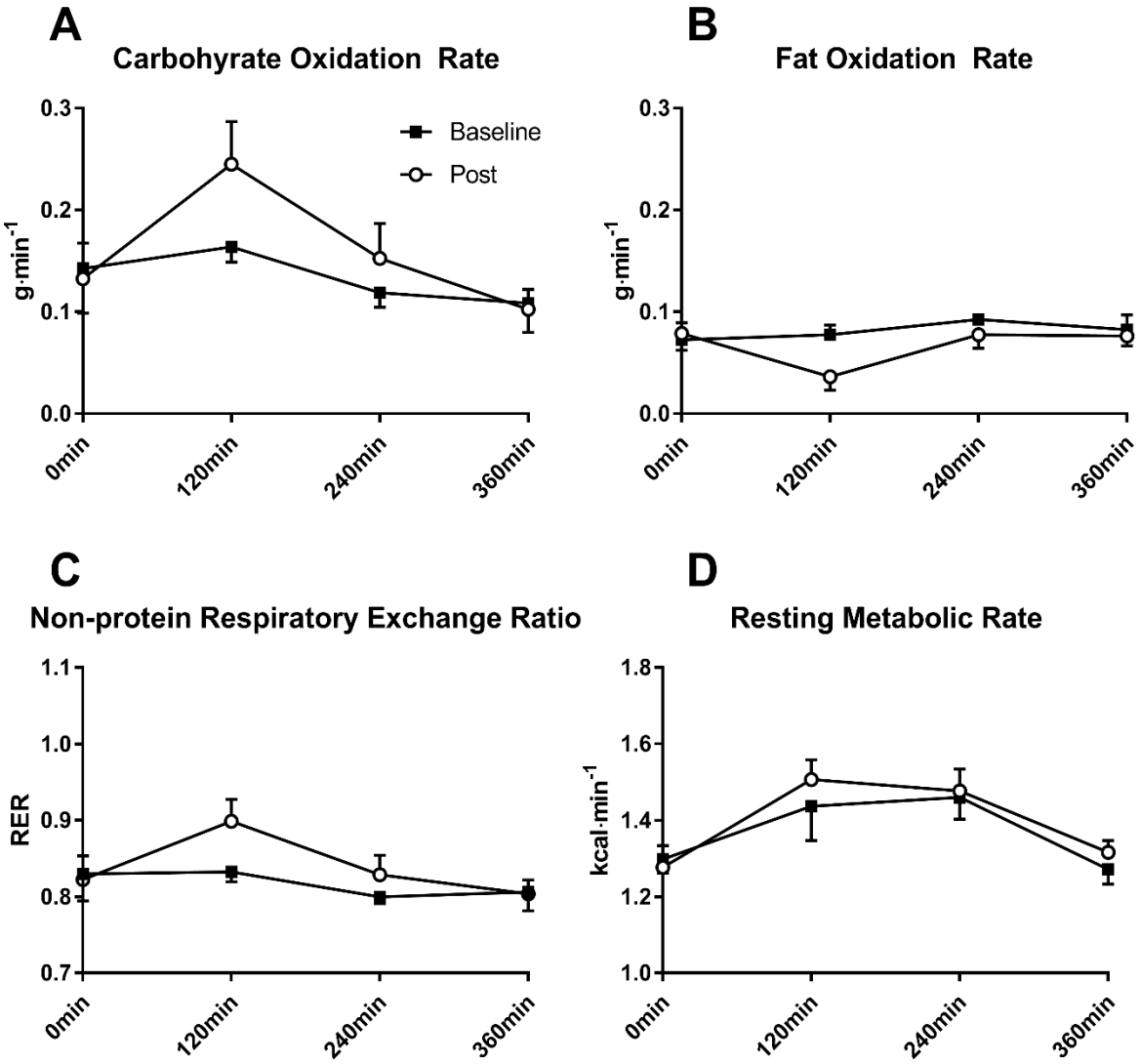


**Figure 11.** Resting rates of carbohydrate (A) and fat oxidation (B), respiratory exchange ratio (C) and metabolic rate (D) prior to and 2, 4 and 6h post meal challenge, before (baseline) and after 16 sessions of HIIT (post). Oxidation rates are corrected for protein oxidation via equations derived from Frayn (1983), using urine and plasma urea concentrations. Resting metabolic rates are determined using the Weir equation (1949).

## 3.4 Blood Biomarkers and Peripheral Blood Flow

**Table 2.** Pre-training (baseline) and Post-training (post) fasted, resting values of blood biomarkers and brachial artery characteristics.

| Fasting Measure | Baseline | Post | *P*-value |
|---|---|---|---|
| Glucose ($mmol \cdot L^{-1}$) | 4.16 (± 0.09) | 4.13 (± 0.20) | 0.89 |
| Triglyceride ($mmol \cdot L^{-1}$) | 0.82 (± 0.10) | 0.87 (± 0.09) | 0.54 |
| Free Fatty Acids ($mmol \cdot L^{-1}$) | 0.53 (± 0.05) | 0.49 (± 0.04) | 0.13 |
| Branched Chain Amino Acids ($\mu mol \cdot L^{-1}$) | 252.12 (± 13.67) | 245.54 (± 14.19) | 0.73 |
| Insulin ($mU \cdot L^{-1}$) | 8.58 (± 0.97) | 10.06 (± 1.18) | 0.30 |
| Brachial Artery Diameter (mm) | 4.09 (± 0.12) | 4.01 (± 0.16) | 0.21 |
| Brachial Artery Blood Flow ($mL \cdot min^{-1}$) | 44.05 (± 5.47) | 68.42 (± 7.84) | 0.09 |

Data presented as mean (± SEM); n=8.

In the fasted, resting state, brachial artery diameter was not different between the pre- and post-training conditions (4.09 ± 0.12 vs. 4.01 ± 0.16mm, $P = 0.21$), while fasted resting forearm blood flow tended to increase after HIIT (44.1 ± 15.5 vs. 68.4 ± 22.1$mL \cdot min^{-1}$, $P = 0.09$). Repeated measures ANOVA revealed no significant main effect of training on forearm blood flow during the tolerance test ($P = 0.14$). A significant effect of time was reported ($P < 0.01$), but no time × condition interaction was found ($P = 0.23$) when data were analysed over the entire 6h period (Fig. 12).

It was apparent however, that 4h post-feeding, blood flow responses after HIIT diverged from those at baseline. When repeated measures ANOVA was performed on hours 4, 5 and 6 only, the main effect approached

statistical significance ($P$ = 0.07). Forearm blood flow tAUC, but not iAUC, tended to increase after training (41.24 ± 2.29 vs. 50.16 ± 5.31L·6h$^{-1}$, $P$ = 0.08, and 25.38 ± 2.99 vs. 25.53 ± 5.88L·6h$^{-1}$, $P$ = 0.98, respectively).

Four weeks of HIIT did not significantly alter fasting arterialised glucose concentration (4.16 ± 0.25 vs 4.13 ± 0.56mmol·L$^{-1}$, $P$ = 0.89). While numerical differences were present during the postprandial period, ANOVA revealed no main effect of HIIT on arterialised blood glucose concentrations ($P$ = 0.34) (Fig. 13; A). A significant effect of time was reported ($P$ < 0.001), but no time × condition interaction was found ($P$ = 0.62). Similarly, no main effect of training was found on arterio-venous differences ($P$ = 0.17) or subsequent glucose uptake ($P$ = 0.11), however both were significantly influenced by time ($P$ < 0.001), but were without interaction ($P$ = 0.93 and $P$ = 0.92, respectively) (Fig. 13; C and D, respectively). Furthermore, neither glucose total (tAUC) nor incremental (iAUC) area under the curve were altered with training (1711 ± 79.5 vs 1673 ± 161mmol·l$^{-1}$·6h$^{-1}$, $P$ = 0.42, and 214 ± 66 vs 186 ± 129mmol·L$^{-1}$·6h$^{-1}$, $P$ = 0.54, respectively).

After HIIT, neither fasting insulin concentration nor HOMA2-IR score were different from those observed at baseline (8.58 ± 2.74 vs 10.06 ± 3.33mU·L$^{-1}$, $P$ = 0.30, and 1.16 ± 0.37 vs 1.37 ± 0.45mU·L$^{-1}$, $P$ = 0.27, respectively) (Fig. 14; B). In the postprandial period, neither tAUC nor iAUC was altered with training (11849 ± 3544 vs 11592 ± 4016mU·L$^{-1}$·6h$^{-1}$, $P$ = 0.76, and 8759 ± 2936 vs 7971 ± 3224mU·L$^{-1}$·6h$^{-1}$, $P$ = 0.33, respectively). ANOVA reported no main effect of HIIT on postprandial insulin concentrations ($P$ = 0.84) (Fig. 14; A). There was a main effect of time on insulin concentration ($P$ < 0.001), but no time × condition interaction ($P$ = 0.84). Paired t-tests revealed the Matsuda Index score (reflecting the first 2h postprandial insulin and glucose responses) not to be different between pre- and post-HIIT (15.90 ± 3.98 vs 15.48 ± 5.06, $P$ = 0.77) (Fig. 14; C).

Fasting arterialised TAG concentration was not different after training (0.82 ± 0.29 vs 0.87 ± 0.26mmol·L$^{-1}$, $P$ = 0.54). In the postprandial state, total

and incremental TAG AUCs were also not altered (561 ± 205 vs 619 ± 248 mmol·L$^{-1}$·6h$^{-1}$, *P* = 0.33, and 265 ± 116 vs 305 ± 172mmol·L$^{-1}$·6h$^{-1}$, *P* = 0.35, respectively). ANOVA revealed no main effect of training on arterialised TAG concentrations (*P* = 0.32), a significant effect of time (*P* < 0.001) and no interaction effect (*P* = 0.87) (Fig. 15; A). Training did not influence arterio-venous differences (*P* = 0.53) (Fig. 15; C) nor subsequent uptake of TAG (*P* = 0.17) (Fig. 15; D) between pre- and post-HIIT, with no effect of time (*P* = 0.79 and *P* = 0.52) nor time × condition interactions (*P* = 0.56 and *P* = 0.38, respectively).

Fasting arterialised FFA concentration was not influenced by training (0.53 ± 0.13 vs 0.49 ± 0.12mmol·L$^{-1}$, *P* = 0.13). Neither total, nor incremental, arterialised FFA AUC over the postprandial period were significantly different between the baseline and post-training state (134 ± 18 vs 126 ± 25mmol·L$^{-1}$·6h$^{-1}$, *P* = 0.12, and -58 ±37 vs -51 ± 38mmol·L$^{-1}$·6h$^{-1}$, *P* = 0.52). ANOVA reported no main effects of HIIT on arterialised postprandial FFA concentrations (*P* = 0.12) (Fig. 16; A), a significant effect of time (*P* < 0.01) and no time × condition interaction effect (*P* = 0.92). While there was an effect of time on arterio-venous differences (*P* < 0.01), ANOVA reported no main effect of training (*P* = 0.18), nor of time × condition (*P* = 0.61) (Fig. 16; C). However, ANOVA did reveal main effects of condition and time on FFA uptake (*P* = 0.03 and *P* < 0.01, respectively), but no interaction between the two (*P* = 0.21) (Fig. 16; D). Similarly, there were subsequent main effects of training and time on FFA extraction (*P* = 0.05 and *P* = 0.01), but not a time × condition interaction (*P* = 0.59) (Fig. 16; E).

Fasting arterialised BCAA concentration was not different before and after training (252 ± 39 vs 246 ± 40µmol·L$^{-1}$, *P* = 0.74). ANOVA revealed no main effect of HIIT on arterialised BCAA concentrations (*P* = 0.41), yielded a significant effect of time (*P* < 0.001), but no interaction effect (*P* = 0.79) (Fig. 17; A). Furthermore, neither tAUC nor iAUC of arterialised BCAA concentrations were different from baseline (109 ± 13 vs. 116 ± 15×10$^{3}$µmol·L$^{-1}$·6h$^{-1}$, and *P* = 0.39, and 18 ± 10 vs. 27 ± 14×10$^{3}$µmol·L$^{-1}$·6h$^{-1}$, *P* = 0.21, respectively). ANOVA reported that neither BCAA arterio-

venous differences, uptake, or extraction exhibited main effects of training or time, nor interaction effects (Fig. 17; C, and D, respectively).

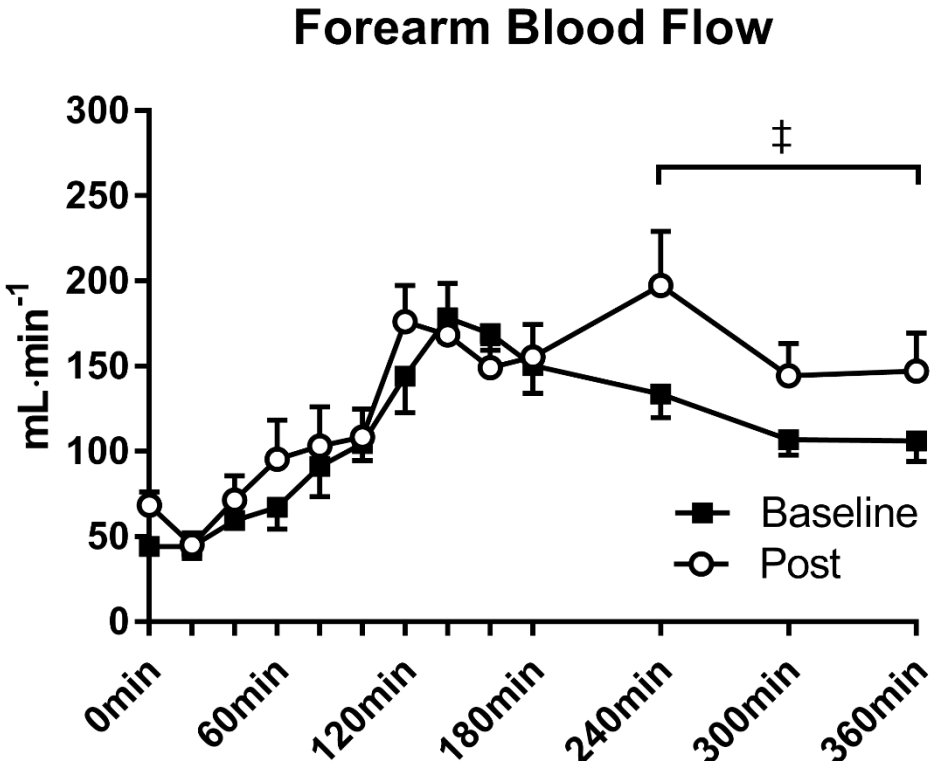


**Figure 12.** Fasted (t=0) and postprandial blood flow through the brachial artery, assessed via Doppler ultrasonography before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8. In the final 3h of the tolerance test, brachial artery blood flow tended to increase after training, ‡*P* = 0.07 (ANOVA main effect).

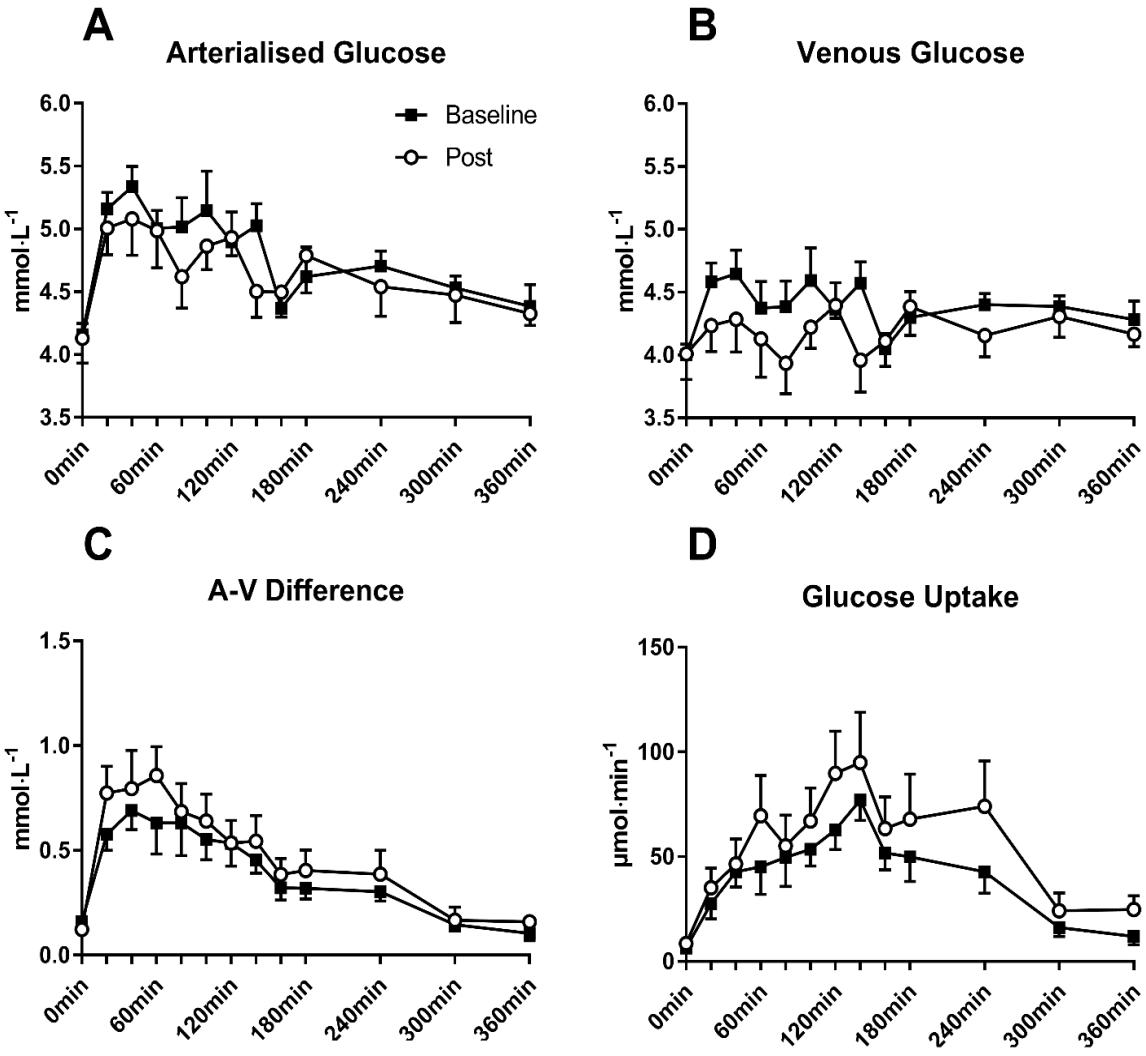


**Figure 13.** Fasted (t=0) and postprandial glucose concentrations (A and B), arterio-venous difference (C) and subsequent rate of uptake, before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8.

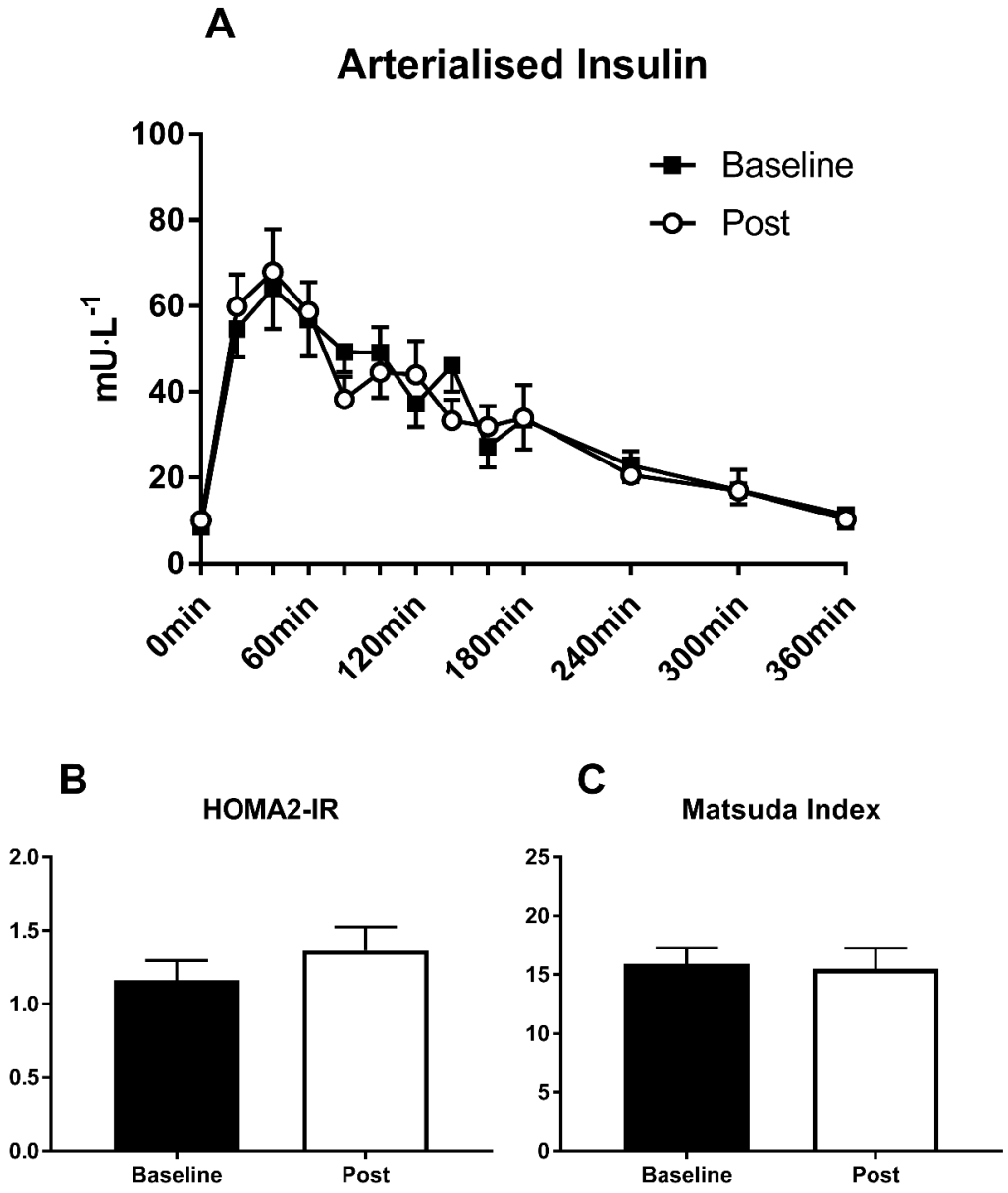


**Figure 14.** Fasted (t=0) and postprandial plasma insulin concentrations (A), HOMA2 insulin resistance (B) and Matsuda Index (derived from insulin and glucose concentrations during fasting and 0-120min after feeding), before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8.

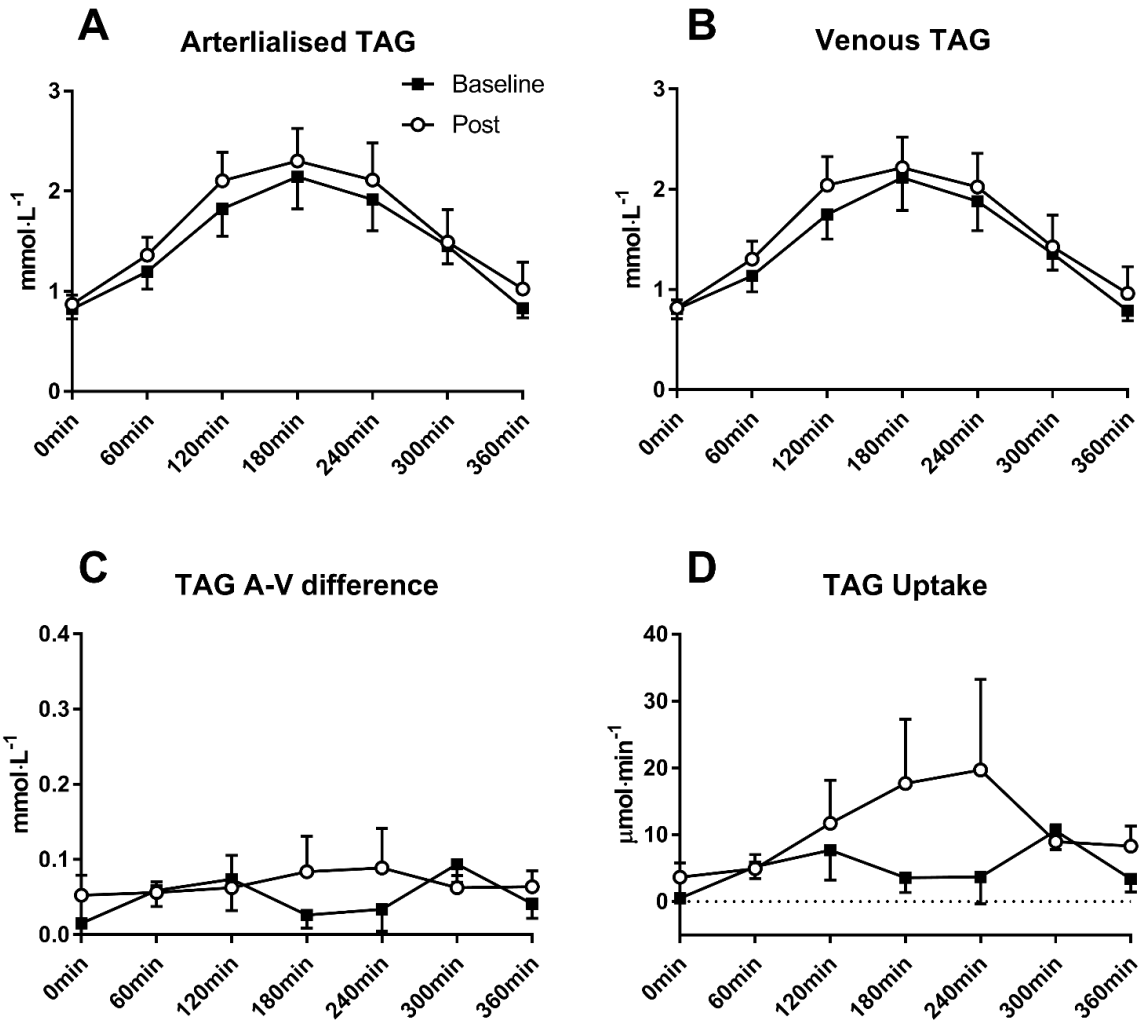


**Figure 15.** Fasted (t=0) and postprandial triglyceride concentrations (A and B), arterio-venous differences (C) and subsequent rate of uptake, before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8.

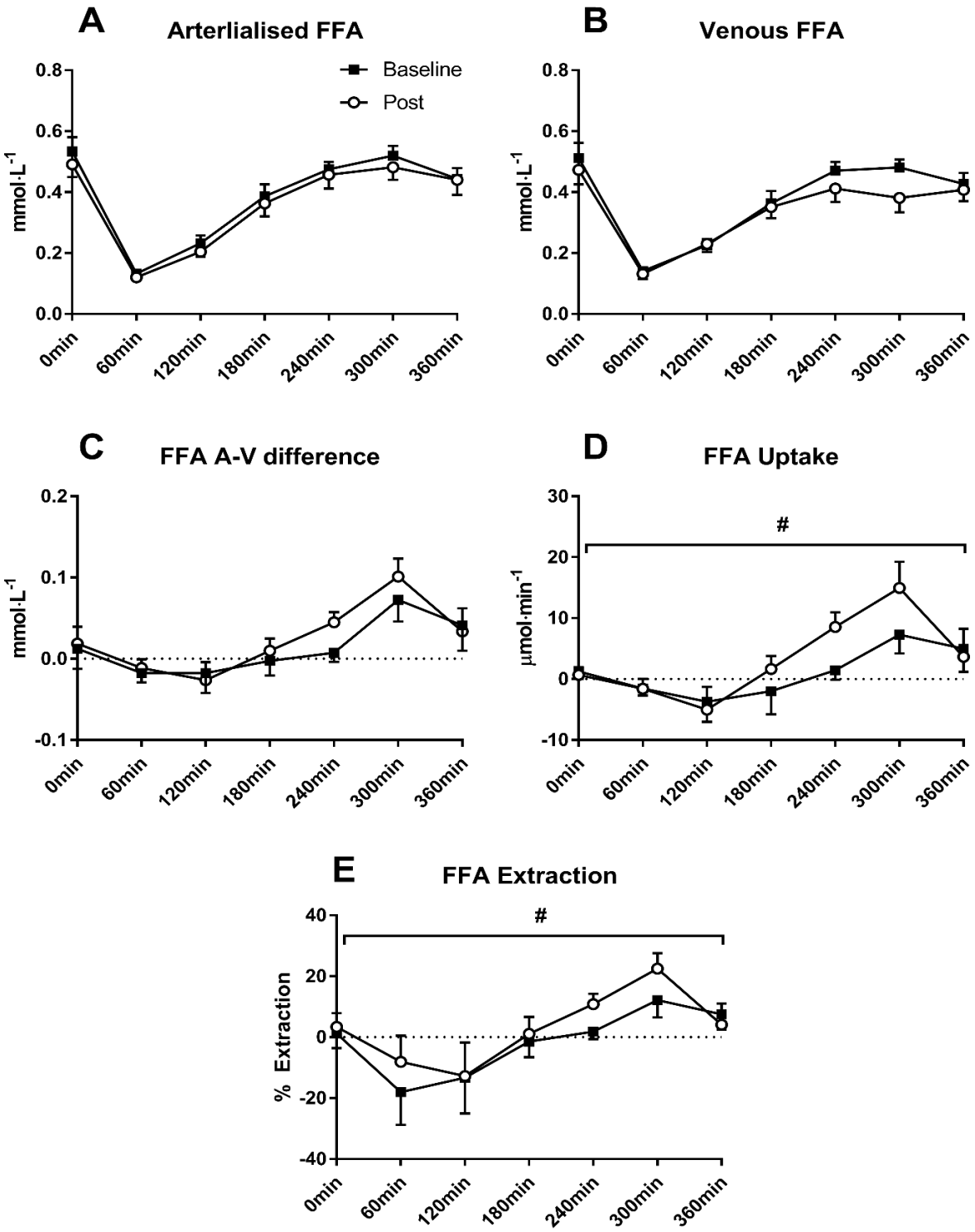


**Figure 16.** Fasted (t=0) and postprandial free fatty acid concentrations (A and B), arterio-venous differences (C), subsequent rate of uptake (D) and fractional extraction (E), before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8. Forearm free fatty acid uptake and extraction significantly increased after training, $^{\#}P < 0.05$ (ANOVA main effect).

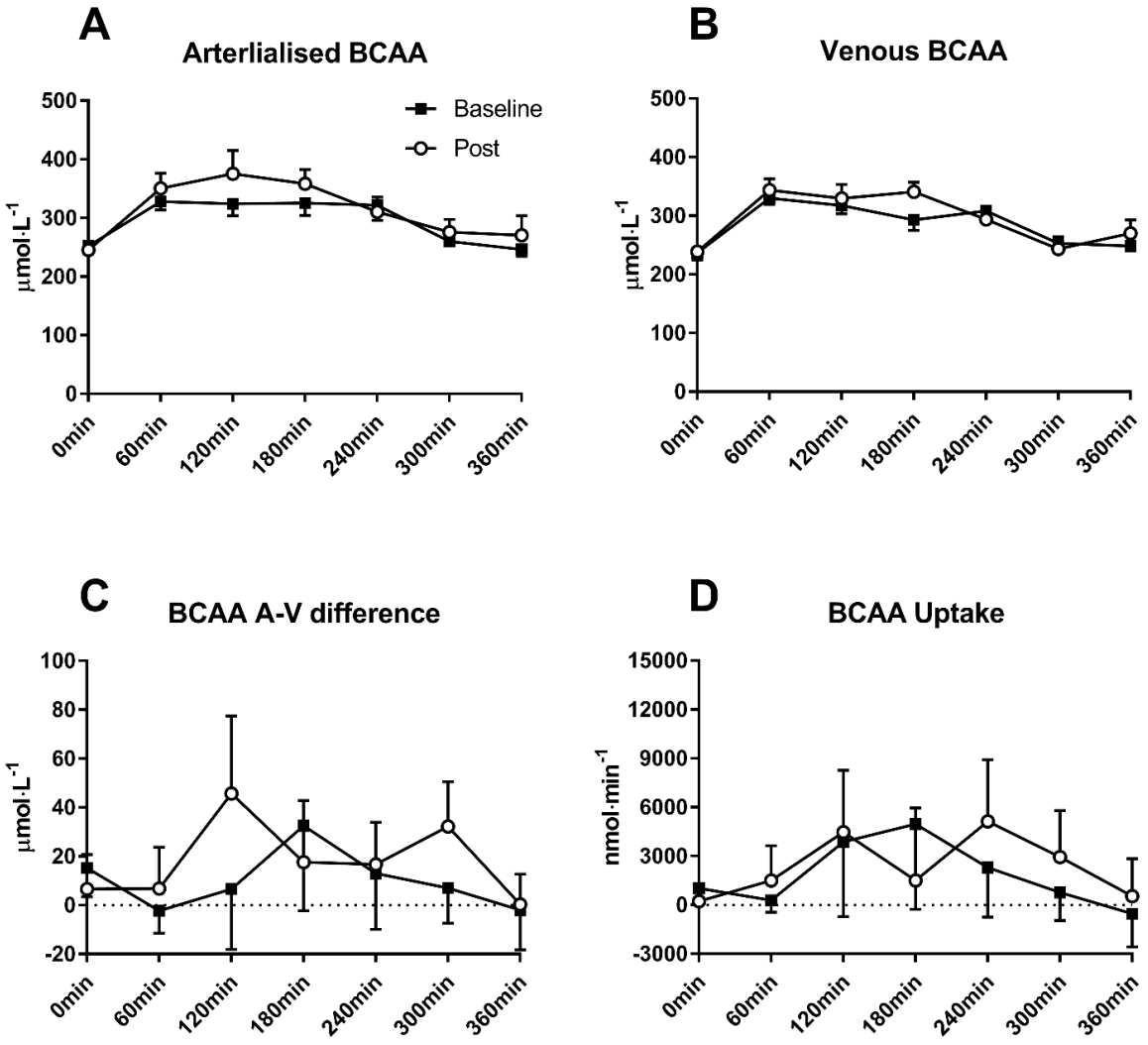


**Figure 17.** Fasted (t=0) and postprandial branched chain amino acid concentrations (A and B), arterio-venous differences (C) and rate of uptake (D), before (baseline) and after 16 sessions of HIIT (post). Data displayed as mean ± SEM; n=8.

## 3.5 Protein Content of Lipolytic Enzymes in Skeletal Muscle

Anti-αactin protein content of VL muscle tissue tended to increase, so proteins of interest were instead normalised against CypB. Two lanes exhibited no visible CypB band, despite good previous bands for anti-αactin on the same membrane, so it was assumed that these lanes experienced poor CypB antibody binding and so were excluded from analysis. Hence, for proteins of interest n=6. There was no significant difference in LPL protein content of the *VL* muscle after 16 sessions of HIIT (2.57 ± 0.37 vs. 1.59 ± 0.51au, $P = 0.11$) (Fig. 18). Similarly, after training, ATGL muscle protein content was also not significantly different from baseline (2.89 ± 0.89 vs. 2.12 ± 0.20au, $P = 0.50$) (Fig. 19).

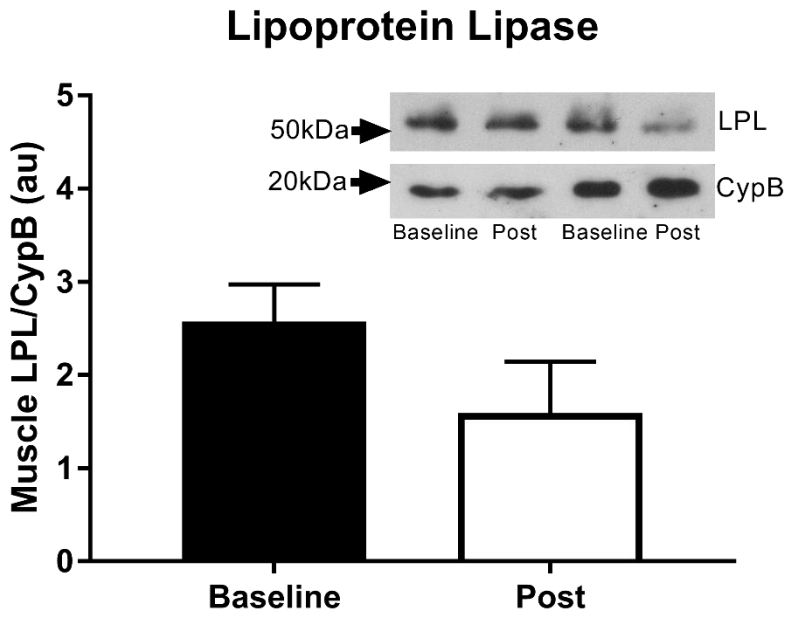


**Figure 18.** Lipoprotein lipase protein content (normalised against cyclophilin B) in vastus lateralis muscle before (baseline) and after 16 sessions of HIIT (post). Muscle samples taken from fasted, resting participants following ≥72h abstinence from exercise. Data displayed as mean ± SEM; n=6. Top: bands of LPL protein at ~52kDa and CypB protein at ~19kDa, at baseline and post-training, in 2 representative participants.

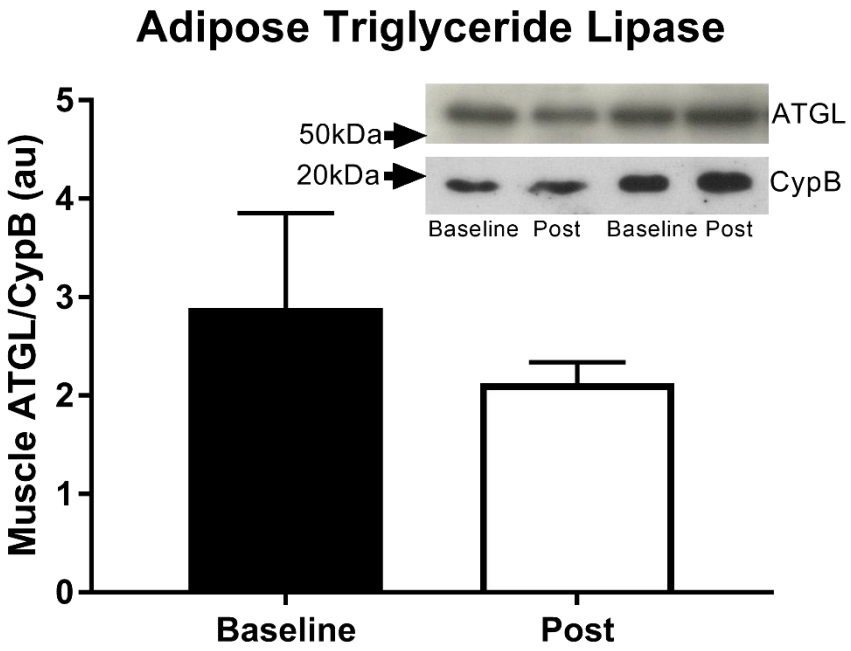


**Figure 19.** Adipose triglyceride lipase protein content (normalised against cyclophilin B) in vastus lateralis muscle before (baseline) and after 16 sessions of HIIT (post). Muscle samples taken from fasted, resting participants following ≥72h abstinence from exercise. Data displayed as mean ± SEM; n=6. Top: bands of ATGL protein at ~55kDa and CypB protein at ~19kDa, at baseline and post-training, in 2 representative participants

# Chapter 4:
# Discussion

## 4.1 Overview of Findings

The primary purpose of this study was to investigate the chronic effects of 16 sessions of HIIT on postprandial metabolism in healthy young males. To the best of our knowledge this study is the first to comprehensively assess metabolic responses to chronic HIIT without weight-loss, beyond the 'transient' period of 12-48h post-exercise.

The principle novel finding of this study was that 16 sessions of HIIT significantly increased postprandial free fatty acid uptake and extraction across the forearm muscles, ≥72h after the final bout of exercise. Furthermore, HIIT resulted in a delayed-onset, but marked increase in postprandial peripheral blood flow, greatly improved cardiopulmonary function as indicated by increased $VO_{2max}$, and significant increases in quadriceps muscle thickness. Contrary to some earlier studies, no significant changes were observed in fasting or postprandial glucose, insulin or TAG concentrations, nor skeletal muscle TAG uptake and protein content of lipases previously associated with the latter.

## 4.2 Lipid and Lipolytic Enzyme Responses

Skeletal muscle FFA uptake and extraction were significantly increased after training, which was most apparent in the latter half of the tolerance test. In accordance with existing literature, FFA uptake in the fasted state and the first 3 postprandial hours, was slightly negative indicating net FFA release by skeletal muscle (Havel, 1998). Following 9 weeks of MICT, Bergman et al. (1999) reported increased postprandial leg net FFA uptake, but not fractional extraction, during exercise at 65% $VO_{2max}$. Following 60h abstinence from exercise, Herd et al. (2000) found endurance trained individuals to exhibit lower postprandial tAUCs for FFA, glucose and insulin,

than their untrained counterparts, however TAG tAUC was not different between groups.

Together, the findings of Herd and colleagues with those of our own, suggest that chronic exercise may moderate postprandial uptake and/or concentrations of FFA after ≥72h exercise abstinence, but that the mechanisms responsible operate independently of those for the transient reductions often found in TAG. Seemingly incongruously, increased FFA uptake has been associated with the insulin resistant state for its role in the accumulation of intracellular lipids, while impaired FFA uptake has been observed in individuals with impaired glucose tolerance (Turcotte & Fisher, 2008; Turpeinen et al., 1999). It is important to note, however, that the deleterious associations of FFA uptake and metabolic health are observed in physically inactive, but not trained, individuals; suggesting that exercise training may alter the fate of circulating FFAs in the postprandial state away from re-esterification into IMTG, and towards increased oxidation within skeletal muscle. Elevated IMTG content is associated with insulin resistance, and it has been shown that reducing intracellular fat increases insulin sensitivity (Heron-Milhavet et al., 2004; Krssak et al., 1999). Conversely, cross-sectional and longitudinal studies typically reveal trained individuals to have greater IMTG content yet exhibit increased insulin sensitivity, a phenomenon termed 'the athlete's paradox' (Goodpaster, He, Watkins, & Kelley, 2001; Russell, 2004). Indeed, Alsted (2009) reported a 28% reduction in IMTG concentration 48h after 8 weeks of intermittent exercise. However, since we reported no change in postprandial substrate oxidation rates, our findings do not suggest the fate of up-taken FFAs was influenced by training.

Despite increased uptake and fractional extraction, we found FFA concentrations to be unchanged after training. Previously, however, 2 weeks of HIIT was shown to reduce FFA uptake from the jejunum by 6%, and by 37% following HIIT and MICT, respectively (Motiani et al., 2017). Our findings therefore do not support the idea that exercise training diminishes intestinal uptake of lipids. Furthermore, reduced gastric uptake of FFA would confound our findings and those of previously reported trends

towards increased skeletal muscle uptake after 2 weeks of HIIT, since reduced appearance of FFA into the circulation, would likely lower their abundance, thus availability, for uptake into skeletal muscle tissue (Eskelinen et al., 2015).

Neither fasting, nor postprandial TAG concentrations were significantly altered following training. Herd et al. (2001) previously found that following 60h abstinence from exercise, neither fasting nor postprandial TAG concentrations were different between trained and untrained individuals. The authors noted that reduced fasting TAG, which is highly associated with postprandial responses, begins to return to pre-exercised levels after ~24h. Similarly, following 24 weeks of training, Crouse et al (1997) found improved fasting lipid profiles ≤48h after exercise, but not when measured >60h. Our findings resemble Bond and colleagues' (2015) who, using an almost identical protocol to the one here employed, found no effect of 2 weeks of HIIT on traditional biomarkers of cardiovascular risk. Contrary to previous findings, Bond also found no effect of HIIT on any biomarker when measured 1-day post-exercise, nor $VO_{2max}$, but did report increased fasting and postprandial brachial artery FMD.

It has been suggested that previously observed exercise-induced TAG reductions may owe to a decreased rate of chylomicron appearance from the gut. Kolifa (2004) fed participants macadamia nuts ~14h after 60min of cycling at 70-75% $HR_{max}$. Using gas chromatography, the authors reported that only macadamia-prevalent fatty acids of plasma TAG were significantly lowered, which may represent attenuated intestinal uptake. Others have claimed that the influence of reduced chylomicron appearance may be overstated (Gill & Hardman, 2003). The postprandial TAG responses reported in this thesis show no indication that the rate of lipid uptake from the gut may have been decreased with training. In line with our findings, it has been reported that pre-prandial exercise does not reduce the time to peak postprandial TAG concentration, nor decelerate gastric emptying (Gill, Frayn, Wootton, Miller, & Hardman, 2001; Gill, Mees, Frayn, & Hardman, 2001). Neither fasting nor postprandial concentrations of apoB48 (a structural component, thus marker, of

chylomicron particle number) have been shown to be reduced by exercise, despite reductions in fasting and postprandial TAG of 16% and 23%, respectively (Anthony P. James, Slivkoff-Clark, & Mamo, 2007). It appears, therefore, that reduced chylomicron formation and appearance may occur following acute exercise, but not to a physiologically meaningful extent.

Another proposed mechanism of TAG reduction is the upregulation of lipolytic enzymes. LPL binds to highly charged heparin sulphate chains on the luminal surface of capillary endothelial cells, extending into the lumen to enable the binding of TAG-rich lipoproteins at the apoC-II binding site (Mead, Irvine, & Ramji, 2002). Once activated, LPL hydrolyses TAG, liberating FFA and 2-monoacyglycerol. (De Man, Cabezas, Van Barlingen, Erkelens, & de Bruin, 1996). Insulin affects the LPL activity of adipose and skeletal muscle tissue differently. In adipose tissue, insulin stimulates LPL production and secretion, which drives TAG hydrolysis, with subsequent uptake, re-esterification and ultimately storage of TAG in adipocytes (Sadur & Eckel, 1982). Conversely, while skeletal muscle contractile activity locally increases LPL activity, insulin has been shown to decrease it (Kiens, Lithell, Mikines, & Richter, 1989a, 1989b).

Following 5 bouts of exercise in 5 consecutive days, skeletal muscle LPL mRNA was increased 4h after the final bout, peaking at ~18h (Seip et al., 1997). Protein content of LPL has been shown to peak 18h after acute exhaustive exercise, and both LPL protein and mRNA content return to baseline within 48h (Kiens & Richter, 1998). Our findings support the insulin-LPL association in as much as indicating that four weeks of HIIT does not result in chronic changes in insulin concentrations nor skeletal muscle LPL content. Herd et al. (2001) reported that 90min of MICT performed ~16h before an OFTT, did not influence fasted skeletal muscle LPL activity, despite a ~38% reduction in TAG tAUC. Of note, despite an absence of change, participants with higher basal LPL activity exhibited greater reductions in PPL, suggesting a more complicated relationship between LPL and TAG clearance during the postprandial period.

Adipose triglyceride lipase (ATGL); an enzyme expressed primarily in human adipose, but also skeletal muscle tissue; plays an essential role in the lipolysis of adipose and intramuscular TAG, but has also been linked to reduced circulating TAG concentrations (Zimmermann et al., 2004). ATGL exhibits high TAG specificity and catalyses the initial step in TAG catabolism by hydrolysing the first ester bond, which produces diglycerides that can be further hydrolysed by hormone sensitive lipase (HSL). To the best of our knowledge, this study is the first to assess LPL and ATGL protein content >48h after chronic HIIT. No change in either was evident in *VL* muscle at rest.

Protein level of ATGL in the fasted state has been shown to remain elevated at 48h post-exercise following an 8-week intermittent training program (Alsted et al., 2009). It is unclear what the consequences of concurrently increased skeletal muscle and/or adipose ATGL and LPL activity in the postprandial state would be, but it could be speculated that they would result in a cycle of re-esterifcation vs. intracellular TAG hydrolysis. LPL-mediated hydrolysis of circulating TAG might drive increased FFA uptake and subsequent re-esterification into adipose and skeletal muscle tissue, while increased ATGL would drive the hydrolysis of intracellular lipids, further increasing skeletal muscle FFA availability, which might explain the substantial increases in postprandial skeletal muscle FFA oxidation that have previously been found ≤48h post-exercise. Conversely, HSL action has been found to decline rapidly after feeding, which may reflect reduced efflux of FFA from adipose tissue in the postprandial state, resulting in net FFA re-esterification (Keith N. Frayn, Coppack, Fielding, & Humphreys, 1995).

The frequent disparity between results in lipaemia-lowering interventions may be attributable to inter-individual differences that arise from apoE genotypic variance (Burns et al., 2015). Specifically, carrying the ε4 allele has been implicated in less favourable lipid profiles and reduced responsiveness to cholesterol-lowering drugs compared to ε2 and ε3 carriers (Zhang et al., 2017). Individuals exhibiting the ε4/4 phenotype tend towards greater fasting and postprandial TAG and chylomicron retinyl

esters responses (Brown & Roberts, 1991). Furthermore, PPL responses following pre-prandial exercise are different between individuals with different apoE genotype. Those carrying the ε2 and ε3 allele experience attenuated PPL after MICT and HIIT, with ε2 individuals experiencing greater responsiveness to MICT than those with ε3, while PPL appears unaltered in ε4 allele carriers (Ferreira et al., 2013). These findings highlight the importance of individually tailored exercise prescription. Genotypic variance in the apoE gene could explain high levels of inter-individual response variance observed in this study (Δfasting and Δpeak TAG ranged from -24 to +75% and -24 to +72%, respectively) and many other investigations. Future studies could benefit from first genotyping participants before undertaking interventions.

Prior exercise has also been shown to increase postprandial skeletal muscle and hepatic blood flow, which may be partly attributable for previously observed TAG reductions by increasing hepatic substrate delivery, hence uptake (Hurren, Balanos, & Blannin, 2011). Since we reported no changes in circulating TAG or FFA concentrations, it seems unlikely that hepatic blood flow or uptake increased. Rather, increased FFA uptake and fractional extraction are more likely indicative of enhanced muscle metabolism and peripheral vascular function.

## 4.3 Brachial Artery and Blood Flow Responses

In the present study, a strong trend towards increased brachial blood flow was observed during the last 3 hours of the postprandial period after training. While we measured blood flow via the brachial artery, it seems likely that since cycling elicits greater femoral than brachial hyperaemia, had we assessed femoral flow we might have observed a greater effect (Gaenzer et al., 2001). Nevertheless, given the remoteness of the brachial artery from the exercising muscles and its relative absence of involvement in cycling, our findings may be indicative of increased whole-body vessel responsiveness.

Raitakari et al. (2000) found strong correlations between brachial artery blood flow and postprandial insulin and TAG concentrations ($r = 0.80$, $P < 0.02$ and $r = 0.77$, $P < 0.005$, respectively), and posited that increased flow was mediated by changes in their concentrations. This appears not to be the case in our participants, since neither fasting nor postprandial insulin concentrations were altered by training, yet brachial flow was increased. Furthermore, we reported that flow was only increased ≥4h after feeding, by which time insulin concentrations both pre- and post-training had almost returned to fasting levels.

Our findings better reflect those of Sawyer et al. (2016) who, following 8 weeks of 10x60s HIIT, found no change in resting BAD, but did observe increased flow mediated dilatation (FMD) (5.13 vs. 8.98%) ~72h after exercise in obese adults. Sawyer's findings were in spite of no significant changes in blood lipids, insulin, glucose, vascular adhesion molecules, nitric oxide availability, or antioxidant capacity, which have previously been suggested to be implicated in increased blood flow responses. Interestingly, the reverse was seen in a MICT group who increased BAD, but not FMD. As noted by the authors, it is difficult to determine whether such findings are the product of structural remodelling of the macro vasculature or of altered vascular tone, by supressing vasoconstrictive or increasing vasodilatory factors.

Exercise training appears to first increase local, then remote, vasodilator activity, followed by outwards arterial remodelling (Thijssen et al., 2010). Increased femoral artery diameter of the exercising leg has previously been identified after 6 weeks of one-legged cycling (Miyachi et al., 2001). Given the 4-week time course of the HIIT intervention employed in this study, the use of a non-training limb to assess blood flow, and the absence of increased BAD, it seems likely that the increased postprandial blood flow reported here predominantly reflects non-morphological changes.

## 4.4 Blood Glucose and Insulin Responses

Despite the well documented association between physical activity and blood glucose homeostasis (Kaizu et al., 2014), inconsistent timing of postprandial assessment after training has resulted in a field of mixed findings. Accordingly, this study helps in part to answer a recent call for glucose monitoring for at least 72h post-exercise (Cassidy, Thoma, Houghton, & Trenell, 2017). Having refrained from physical activity for 72h after the final bout of HIIT, participants of this study did not exhibit any change in fasting glucose, postprandial concentrations, nor AUCs. The same was true for arterio-venous differences, uptake, and extraction of glucose across the forearm. Similarly, fasting and postprandial insulin concentrations were not influenced by chronic HIIT, and there were no changes in HOMA2-IR or Matsuda index.

A recent meta-analysis of the effects of chronic HIIT on glucose regulation and insulin resistance was unable to analyse postprandial responses due to insufficient data to perform meaningful comparisons (Jelleyman et al., 2015). While exercise undoubtedly acutely reduces glycaemia in many populations, the duration of such effects appears to be somewhat fleeting and there currently exists limited literature to compare our findings in healthy young males to.

Nevertheless, 2-3 days after the concluding bout of just 6 sessions of HIIT (comprising 4-6x30s cycle sprints) untrained healthy young men exhibited reduced tAUC for plasma glucose, insulin, and FFA in response to an OGTT, despite an absence of change in their fasting concentrations (though FFA did tend to reduce) (Babraj et al., 2009). Similar results were found following the completion of 12 weeks of either MICT of SIT in sedentary young males. Gillen et al. (2016) noted similarly improved insulin sensitivity index scores and reduced glucose tAUCs during an OGTT with both exercise modalities, despite no changes in fasting plasma glucose, insulin, or insulin tAUC. It seems likely that the participants in our study would have exhibited lower postprandial glucose and insulin responses for ≤48h, but their absence at 72h mirrors findings of increasing postprandial

glucose and insulin responses reported after just 3 days of reduced physical activity (<5000 steps·day$^{-1}$) in habitually active young adults (≥10000 steps·day$^{-1}$) (Mikus et al., 2012).

Of the few studies to have investigated the effect of interval training on glycaemia beyond the transient post-exercise period, some positive findings have been reported in obese, diabetic subjects. Eight weeks of training using a 10x60s protocol, reduced postprandial glucose concentrations at some, but not all, time points during a 2h OGTT, in middle aged type II diabetics, but not healthy controls, when assessed after 2-3 days of avoiding strenuous physical activity (Madsen, Thorup, Overgaard, & Jeppesen, 2015). However, these effects were seen concurrent to an 18% reduction in abdominal fat mass in the diabetics, compared to a 10% reduction in the controls.

Karstoft and colleagues (2013) undertook a randomised controlled trial to compare the effects of 4-months of energy-matched continuous (55% peak energy expenditure) versus intermittent (3min intervals; >70% peak energy expenditure) walk training on glycaemic control in type II diabetics, using continuous glucose monitoring. Only the intermittent group had reduced mean and peak glucose concentrations across the 48-72h post-exercise period. However, the same participants also reduced total body and fat mass, though ANCOVA suggested this accounted for ≤25% of the observed effect. Despite weight-loss and reduced fasting insulin, fasting glucose and postprandial responses to an OGTT, were not significantly lower when assessed 96-120h post-exercise. Conversely, Stensvold (2010) found fasting plasma glucose to be unchanged 48h after completion of 12 weeks of aerobic interval training in middle aged, obese adults with the metabolic syndrome. Further, 48-72h after 10 sessions of exercise, Robinson et al. (2015) reported reduced fasting glucose only with MICT in sedentary adults, but neither MICT nor HIIT impacted fasting insulin or HOMA-IR.

Of note, participants in this study exhibited healthily low fasting glucose and insulin concentrations (4.2 ± 0.1mmol·L$^{-1}$ and 8.6 ± 1.0mU·L$^{-1}$,

respectively) at baseline, followed by modest glucose AUCs. High protein or high fat meals have been shown to elicit substantially lower glucose responses than those of high CHO (Potter et al., 1989). Furthermore, high fat feeding increases time to peak glucose and the addition of fermentable fibre slows CHO absorption from the gut (K. C. Maki, Phillips-Eakley, & Smith, 2016; Shin, Park, & Choue, 2009). In a comprehensive review, Batacan (2017) concluded that ≥12 weeks of HIIT reduced fasting glucose concentration in overweight and obese individuals, but its effect on normal weight populations was unclear. At baseline, participants in this study presented peak postprandial glucose and insulin concentrations of 5.8 ± 0.2mmol·$L^{-1}$ and 78.6 ± 8.1mU·$L^{-1}$ at baseline, respectively. Had we have provided a greater glycaemic load, such as an OGTT (75g glucose) which has been shown to produce glucose and insulin peaks of ~7.9mmol·$L^{-1}$ and ~125mU·$L^{-1}$ in a similar population with comparable fasting concentrations, the heightened response might have increased the relative change, and possibly statistical power, of the trends we observed towards increased glucose arterio-venous differences and uptake (Nurnberger, Dammer, Philipp, Wenzel, & Schafers, 2003).

The mechanisms responsible for supressed glycaemia after HIIT is not well understood, but it has been shown that activation of 5’ adenosine monophosphate-activated protein kinase (AMPK), which occurs with skeletal muscle contractile activity, increases skeletal muscle glucose uptake by increasing translocation of the insulin-regulated glucose transporter 4 (GLUT4) protein (Little et al., 2011; Towler & Hardie, 2007). Indeed, in male diabetic mice (db/db), 10 weeks of HIIT resulted in lower fasting glycaemia than MICT, which was accompanied by greater GLUT4 content and insulin-stimulated protein kinase B (PKB) phosphorylation (Chavanelle et al., 2017). Moreover, regular exercise has been shown to reduce fasting and glucose-stimulated insulin levels by reducing secretion, which may reflect concurrent declines of proinsulin (the prohormone precursor to insulin) and glucokinase (a glucose phosphorylating enzyme that functions as a glucose sensor to pancreatic β-cells) mRNA expression in the pancreas (Koranyi, Bourey, Slentz, Holloszy, & Permutt, 1991).

Declining proinsulin mRNA implies a reduction in insulin synthesis, while a downregulation of glucokinase might decrease β-cell glucose sensitivity (Sigal, Kenny, Wasserman, & Castaneda-Sceppa, 2004).

In an extension of an aforementioned study (Kristian Karstoft et al., 2013), intermittent walk training increased insulin sensitivity index, peripheral glucose disposal and disposition index (a marker of β-cell function), but not insulin secretion, during a hyperglycaemic clamp 6-7 days post-exercise (K. Karstoft et al., 2014). Conversely, glucose, but not insulin AUC has been found to improve after 8 weeks of SIT, despite a significant improvement in HOMA β-cell index in healthy young subjects (Sandvei et al., 2012).

Clearly, the relationship between insulin secretion; sensitivity; concentration; and glycaemia, in the absence of weight-loss, is a complex one that warrants further mechanistic investigation. Chronic HIIT may improve glycaemic and insulinaemic control more than acute exercise, but this effect rapidly decays with time and is inversely proportional to pre-training metabolic health, which may explain our findings of an absence of change ≥72h post-exercise in healthy young males. More substantial effects can be observed in the metabolically impaired, for whom HIIT or AIT may offer a greater and more enduring advantage over MICT. Collectively, however, present research emphasises the importance of regular exercise for managing and improving glycaemia and insulinaemia.

## 4.5 Circulating Branched Chain Amino Acid Responses

Branched chain amino acids, comprising leucine, isoleucine and valine, are both direct and indirect nutrient signals that are essential for the regulation of protein synthesis and degradation. The implication of heightened BCAA concentrations in metabolic health remains contested, with findings suggesting both protective and degenerative effects. Following 16 sessions of HIIT, we report that neither fasting nor postprandial BCAA

concentrations, AUCs, arterio-venous differences, forearm uptake, or extraction were different from baseline.

Howarth et al. (2007) reported that 6 weeks of HIIT and MICT reduced branched-chain oxoacid dehydrogenase complex activation (BCOAD; an insulin-stimulated, rate-determining enzyme for BCAA oxidation (Frick & Goodman, 1989)), which was associated with increased BCOAD kinase protein content. While exercise training appears to enhance the capacity of skeletal muscle for BCAA oxidation, our findings suggest that it does not influence the postprandial uptake or disposal of circulating BCAAs within that tissue.

Six weeks of leucine supplementation has been shown to increase serum adiponectin (a protein implicated in the modulation of glucose and lipid metabolism) and to decrease cholesterol, in rats without weight-loss (Chandran, Phillips, Ciaraldi, & Henry, 2003; Torres-Leal et al., 2011). Contrary to the role of dietary BCAA consumption, increased circulating BCAA concentration has been associated with insulin resistance and type II diabetes (Yoon, 2016). Furthermore, BCAAs and their derivatives have been shown to inversely correlate with insulin sensitivity and the HOMA-IR index (Newgard, 2012). In a 12 year study of 2422 normoglycemic individuals, of whom 201 developed diabetes, BCAAs and aromatic acids concentrations were highly predictive of future diabetes, with individuals in the top quartile experiencing a five-fold risk (Wang et al., 2011).

High BCAA concentrations activate mTOR complex 1, which results in downstream activation of S6 kinase, in turn phosphorylating insulin receptor substrate 1 (IRS-1), which can inhibit insulin signalling (Chen & Yang, 2015; Manning, 2004). Whether this substantially occurs with physiological concentrations of BCAAs is unclear. While our study offers limited insight, the effects of exercise training on circulating BCAAs and their implications for insulin sensitivity/resistance warrants further investigation, to determine any therapeutic potential.

## 4.6 Resting Substrate Oxidation and Energy Expenditure

Indirect calorimetry was used to assess fasting and postprandial substrate oxidation rates, which were adjusted for protein oxidation. There was no change in fasting substrate utilisation, nor a main effect of training on the full 6h time course of assessment. Expectedly, then, no effect of training was seen on RER. Furthermore, fasting RMR was not elevated 72h post-HIIT, and training had no effect on postprandial RMR. Our findings are consistent with Schubert's (2017) following 4 weeks of 10x60s HIIT at 90% peak power output, with 60s rest intervals. The authors reported no change in fasting RMR (though trended towards a 2% increase, $P = 0.06$), RER, or CHO and fat oxidation rates 48-72h post-exercise. Previously, 2 weeks of HIIT (4-6x30s max effort cycling, with 4.5min rest intervals) was shown to increase fat and decrease CHO oxidation at rest 24h, but not 72h, after the final bout (Whyte, Gill, & Cathcart, 2010).

It was previously shown that endurance training increases fat oxidation during exercise by up to 10% following just 6 sessions of HIIT (Roberson, Astorino, Allen, Trost, & Jurancich, 2010). Pre-prandial MICT shifts postprandial substrate utilisation towards fat predominance, but does not alter RMR, while HIIT acutely increases fasting CHO and postprandial fat oxidation (Alkahtani, 2014; Stiegler, Sparks, & Cunliffe, 2008). Given the association of impaired postprandial fat oxidation with obesity and suggestions that insulin mediates increased lipolysis and subsequent fat utilisation; improvements in postprandial fat oxidation may be related to increased insulin sensitivity and metabolic health (Blaak et al., 2006; Schiffelers, Saris, & van Baak, 2001; Stiegler et al., 2008). These findings collectively suggest that short-term exercise training has little enduring effect on substrate oxidation at rest and that increased fat oxidation during exercise primarily represents blood glucose and IMTG sparing, in an attempt to better sustain endurance exercise performance. Indeed, after 48h of exercise abstinence following the completion of 9 months of progressively overloading MICT, despite increasing RMR, neither fat nor

CHO oxidation were altered in young adults in the resting, fasted state (Potteiger, Kirk, Jacobsen, & Donnelly, 2008).

## 4.7 Cardiopulmonary Responses

Maximal oxygen uptake increased by 10.2 ± 7.7% with training. Improvement in $VO_{2max}$ was universal, ranging from +0.3 to +23% (0.14–9.08mL·$O_2$·$kg^{-1}$·$min^{-1}$ in absolute terms). We further investigated individual cardiopulmonary responses, revealing a strong negative correlation between baseline $VO_{2max}$ and Δ%$VO_{2max}$, highlighting greatest HIIT efficacy in those with lower baseline fitness.

Considering the relative brevity of the intervention, these changes represent substantial improvements in cardiopulmonary function. The improvements seen in the participants of this study are akin to those of Sugawara et al. (2001) who reported +12.6% $VO_{2max}$ in participants highly comparable to those of this study (healthy males; 20.1 ± 1.9 years; 65 ± 7.3kg; baseline $VO_{2max}$ 44.5 ± 5.8mL·$kg^{-1}$·$min^{-1}$). Sugawara's intervention, however, employed 8 weeks of MICT of 1h duration at 70% $VO_{2max}$, equating to approximately four times the total exercise duration completed in this study. As previously reported in active boys and sedentary middle-aged males, the 10x60s HIIT protocol was well tolerated and represents a feasible, time-efficient intervention to enhance cardiopulmonary function (Hood et al., 2011; Thackray et al., 2013).

## 4.8 Hypertrophic Response to Training

Muscle thickness (MT) of the *vastus lateralis* and *rectus femoris*, but not the *vastus intermedius* was significantly increased after 16 sessions of HIIT by 12.4 ± 4.2 and 6.9 ± 1.9%, respectively. Historically, endurance exercise was assumed to be non-impacting, or even deleterious to skeletal muscle hypertrophy. Indeed, high volume endurance training has been shown to mitigate the hypertrophic effects of resistance training in athletes (Ronnestad, Hansen, & Raastad, 2012). Despite the enduring dogma, it has long since been shown that MICT can elicit meaningful increases in muscle CSA of older men (Schwartz et al., 1991).

More recently, 4 weeks of cycling HIIT, but not MICT, has been reported to increase quadriceps MT in hockey players by 2.1%, suggesting anabolic/hypertrophic potential of HIIT even in trained individuals (de Souza et al., 2014). In-line with our findings, 10 weeks of running HIIT resulted in an 11% increase in VL CSA of active students (2 male, 10 female), while just 3 weeks of SIT increased VL CSA by ~14% in obese men and women (Blue, Smith-Ryan, Trexler, & Hirsch, 2017; Estes et al., 2017).

Aerobic exercise improves skeletal muscle anabolic sensitivity to insulin, which helps to redress the balance between muscle protein synthesis and breakdown; the chronic imbalance of which can cause muscular atrophy and drive sarcopenia (Fujita et al., 2007). Importantly, 10-weeks of HIIT (45min of 30s cycling at 124% peak work rate interspersed with 30s rest) elicited ~25% increases in CSA of type I and IIa muscle fibres in elderly, advanced COPD patients (16 male, 3 female) (Sipila, Elorinne, Alen, Suominen, & Kovanen, 1997). Conversely, 18-weeks of MICT did not alter the CSA of VL muscle fibres in elderly women (Vogiatzis et al., 2005). The positive impact of HIIT on muscle mass and CSA appears to be greater and more consistent than MICT, which may reflect recent findings that HIIT but not MICT increases myofibrillar and sarcoplasmic protein fractional synthesis rate for at least 48h and 24h, respectively (Bell, Seguin, Parise, Baker, & Phillips, 2015). Therefore, HIIT may stimulate skeletal muscle hypertrophy by increasing anabolic sensitivity and protein synthesis rate, which could offer therapeutic benefits to older populations, where MICT may not.

## 4.9 Limitations

While every effort was made to standardise the conditions of the study, it was not without temporal and financial limitations. Firstly, participants self-recorded diet diaries; the quality of which varied, with some participants providing detailed and consistent information as requested, and others providing limited details (ie. 'two pork sausages'). Subsequently, some analysis was performed using 'average' portion sizes, which may not be

entirely accurate. Furthermore, dietary intake is almost universally underreported. Ideally, we would have provided 3 days of standardised food and drink, but this was beyond the financial scope of the study.

The temporal structure of the study was adhered to with generally good success, however staff, participant, and laboratory availability made scheduling of visits challenging at times. One participant completed all 16 HIIT sessions in just 20 days (though experienced the greatest relative increase in $VO_{2max}$), while another undertook the post-HIIT CPET 149h after the final session, during which a small degree of detraining may have occurred.

Lastly, participants' weight did not change with training, however small increases in quadriceps muscle thickness were found. Since body composition was not assessed before and after the intervention, it cannot be determined whether there was a net increase and/or decrease in muscle mass and/or fat mass, respectively. As such, dual x-ray absorptiometry data could have made a valuable contribution.

# Chapter 5:
# Conclusions

## 5.1 Summary

Four weeks of high intensity interval training without weight-loss amplifies the effective duration of some but not all metabolic improvements associated with acute exercise, in healthy young males. Namely, HIIT increases the postprandial skeletal muscle uptake and extraction of free fatty acids from the circulation, 72h post-exercise. This jointly reflects trends towards augmented postprandial peripheral blood flow and increased arterio-venous differences, but was non-impacting on whole body substrate oxidation rates. Despite previous, confounding associations of increased FFA uptake with insulin sensitivity and resistance, we observed no changes in indices of insulin sensitivity in both the fasted and postprandial state, suggesting adaptations via insulin-independent pathways.

We neither observed changes in circulating triglycerides markers, nor the skeletal muscle protein content of lipoprotein lipase or adipose triglyceride lipase, suggesting no cumulative effect of HIIT on the upregulation of lipolytic activity and/or suppression of TAG. Ergo; TAG-reduction is a transient effect of the last bout of exercise. Similarly, no changes were observed in circulating glucose, insulin, or branched chain amino acids. In the absence of weight loss, HIIT-induced improvements in lipaemia and glycaemia are largely diminished within 72h. The HIIT protocol was well tolerated and proved to be a time-efficient strategy for improving cardiopulmonary function. Furthermore, we reported increases in muscle thickness of the *vastus lateralis* and *rectus femoris*, which may be indicative of a hypertrophic response to the training protocol. Collectively, our findings emphasise the effectiveness of HIIT as a training stimulus, but highlights the importance of regular exercise for the maintenance of benefits to metabolic health.

## 5.2 Future Directions

Although our findings show limited support for enduring effects of HIIT on postprandial metabolism, it is important to recall the good health of our participants at baseline. The improvements seen in FFA uptake, extraction, and peripheral blood flow would likely be amplified in populations presenting with, or at risk of, the metabolic syndrome. Accordingly, future research should employ similar exercise protocols in longitudinal studies of clinically-relevant populations.

To better understand the mechanisms responsible for such changes, and the tissues upon which they act, future studies should assess the activity/protein content of lipolytic enzymes (LPL, ATGL, etc.) and factors involved in insulin signalling (IRS-1, PKB, etc.), in both skeletal muscle and adipose tissue. Lastly, we employed an energy-dense mixed-meal breakfast that provides a reasonable model of the western diet. A shift towards the use of habitual eating with continuous glucose monitoring would further increase the clinical relevance of findings, though caution must be taken to enable sufficient standardisation.

# References


Aamot, I. L., Karlsen, T., Dalen, H., & Stoylen, A. (2016). Long-term Exercise Adherence After High-intensity Interval Training in Cardiac Rehabilitation: A Randomized Study. *Physiotherapy Research International, 21*(1), 54-64. doi:10.1002/pri.1619

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

Aldred, H. E., Perry, I. C., & Hardman, A. E. (1994). The effect of a single bout of brisk walking on postprandial lipemia in normolipidemic young adults. *Metabolism: Clinical and Experimental, 43*(7), 836-841.

Alkahtani, S. (2014). *The effect of moderate- and high-intensity interval training on substrate oxidation and nutrient preferences in obese men*. Paper presented at the Proceedings of the Eleventh International Society of Sports Nutrition (ISSN) Conference and Expo.

Allen, E., Gray, P., Kollias-Pearson, A., Oag, E., Pratt, K., Henderson, J., & Gray, S. R. (2014). The effect of short-duration sprint interval exercise on plasma postprandial triacylglycerol levels in young men. *Journal of Sports Sciences, 32*(10), 911-916. doi:10.1080/02640414.2013.865254

Aloulou, I., Brun, J. F., & Mercier, J. (2006). Evaluation of insulin sensitivity and glucose effectiveness during a standardized breakfast test: comparison with the minimal model analysis of an intravenous glucose tolerance test. *Metabolism: Clinical and Experimental, 55*(5), 676-690. doi:10.1016/j.metabol.2006.01.002

Alsted, T. J., Nybo, L., Schweiger, M., Fledelius, C., Jacobsen, P., Zimmermann, R., . . . Kiens, B. (2009). Adipose triglyceride lipase in human skeletal muscle is upregulated by exercise training. *American Journal of Physiology-Endocrinology and Metabolism, 296*(3), E445-E453. doi:10.1152/ajpendo.90912.2008

Annuzzi, G., Jansson, E., Kaijser, L., Holmquist, L., & Carlson, L. A. (1987). Increased removal rate of exogenous triglycerides after prolonged exercise in man: time course and effect of exercise duration. *Metabolism: Clinical and Experimental, 36*(5), 438-443.

Armstrong, M. L., Heistad, D. D., Marcus, M. L., Megan, M. B., & Piegors, D. J. (1985). Structural and hemodynamic response of peripheral arteries of macaque monkeys to atherogenic diet. *Arteriosclerosis, 5*(4), 336-346.

Athenstaedt, K. (2010). Neutral Lipids in Yeast: Synthesis, Storage and Degradation. In K. N. Timmis (Ed.), *Handbook of Hydrocarbon and Lipid Microbiology* (pp. 471-480). Berlin, Heidelberg: Springer Berlin Heidelberg.

Austin, G. L., Ogden, L. G., & Hill, J. O. (2011). Trends in carbohydrate, fat, and protein intakes and association with energy intake in normal-weight, overweight, and obese individuals: 1971-2006. *American Journal of Clinical Nutrition, 93*(4), 836-843. doi:10.3945/ajcn.110.000141

Babraj, J. A., Vollaard, N. B., Keast, C., Guppy, F. M., Cottrell, G., & Timmons, J. A. (2009). Extremely short duration high intensity interval training substantially improves insulin action in young healthy males. *BMC Endocrine Disorders, 9*, 3. doi:10.1186/1472-6823-9-3

Badimon, L., & Vilahur, G. (2012). LDL-cholesterol versus HDL-cholesterol in the atherosclerotic plaque: inflammatory resolution versus thrombotic chaos. *Annals of the New York Academy of Sciences, 1254*, 18-32. doi:10.1111/j.1749-6632.2012.06480.x

Bansal, S., Buring, J. E., Rifai, N., Mora, S., Sacks, F. M., & Ridker, P. M. (2007). Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA, 298*(3), 309-316. doi:10.1001/jama.298.3.309

Barath, P., Fishbein, M. C., Cao, J., Berenson, J., Helfant, R. H., & Forrester, J. S. (1990). Tumor necrosis factor gene expression in human vascular intimal smooth muscle cells detected by in situ hybridization. *The American Journal of Pathology, 137*(3), 503-509.

Bartlett, J. D., Close, G. L., MacLaren, D. P., Gregson, W., Drust, B., & Morton, J. P. (2011). High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: implications for exercise adherence. *Journal of Sports Sciences, 29*(6), 547-553. doi:10.1080/02640414.2010.545427

Batacan, R. B., Jr., Duncan, M. J., Dalbo, V. J., Tucker, P. S., & Fenning, A. S. (2017). Effects of high-intensity interval training on cardiometabolic health: a systematic review and meta-analysis of intervention studies. *British Journal of Sports Medicine, 51*(6). doi:10.1136/bjsports-2015-095841

Bell, K. E., Seguin, C., Parise, G., Baker, S. K., & Phillips, S. M. (2015). Day-to-Day Changes in Muscle Protein Synthesis in Recovery From Resistance, Aerobic, and High-Intensity Interval Exercise in Older Men. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences, 70*(8), 1024-1029. doi:10.1093/gerona/glu313

Bellou, E., Magkos, F., Kouka, T., Bouchalaki, E., Sklaveniti, D., Maraki, M., . . . Sidossis, L. S. (2013). Effect of high-intensity interval exercise on basal triglyceride metabolism in non-obese men. *Applied Physiology, Nutrition, and Metabolism. Physiologie Appliquée, Nutrition et Métabolisme, 38*(8), 823-829. doi:10.1139/apnm-2012-0468

Bellou, E., Siopi, A., Galani, M., Maraki, M., Tsekouras, Y. E., Panagiotakos, D. B., . . . Sidossis, L. S. (2013). Acute effects of exercise and calorie restriction on triglyceride metabolism in women. *Medicine and Science in Sports and Exercise, 45*(3), 455-461. doi:10.1249/MSS.0b013e318278183e

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2012). *Biochemistry*. Basingstoke: W.H. Freeman.

Bergman, B. C., Butterfield, G. E., Wolfel, E. E., Casazza, G. A., Lopaschuk, G. D., & Brooks, G. A. (1999). Evaluation of exercise and training on muscle lipid metabolism. *American Journal of Physiology-Endocrinology and Metabolism, 276*(1), E106-E117.

Blaak, E. E., Hul, G., Verdich, C., Stich, V., Martinez, A., Petersen, M., . . . Consortium for Nutr-Gene, I. (2006). Fat oxidation before and after a high fat load in the obese insulin-resistant state. *Journal of Clinical Endocrinology & Metabolism, 91*(4), 1462-1469. doi:10.1210/jc.2005-1598

Blue, M. N. M., Smith-Ryan, A. E., Trexler, E. T., & Hirsch, K. R. (2017). The effects of high intensity interval training on muscle size and quality in overweight and obese adults. *Journal of science and medicine in sport*. doi:10.1016/j.jsams.2017.06.001

Bond, B., Cockcroft, E. J., Williams, C. A., Harris, S., Gates, P. E., Jackman, S. R., . . . Barker, A. R. (2015). Two weeks of high-intensity interval training improves novel but not traditional cardiovascular disease risk factors in adolescents. *American Journal of Physiology: Heart and Circulatory Physiology, 309*(6), H1039-1047. doi:10.1152/ajpheart.00360.2015

Bond, B., Gates, P. E., Jackman, S. R., Corless, L. M., Williams, C. A., & Barker, A. R. (2015). Exercise intensity and the protection from postprandial vascular dysfunction in adolescents. *American Journal of Physiology: Heart and Circulatory Physiology, 308*(11), H1443-1450. doi:10.1152/ajpheart.00074.2015

Bond, B., Williams, C. A., Isic, C., Jackman, S. R., Tolfrey, K., Barrett, L. A., & Barker, A. R. (2015). Exercise intensity and postprandial health outcomes in adolescents. *European Journal of Applied Physiology, 115*(5), 927-936. doi:10.1007/s00421-014-3074-8

Botham, K. M. (2008). Oxidation of chylomicron remnants and vascular dysfunction. *Atheroscler Suppl, 9*(2), 57-61. doi:10.1016/j.atherosclerosissup.2008.05.009

Boyle, J. J. (2005). Macrophage activation in atherosclerosis: pathogenesis and pharmacology of plaque rupture. *Current Vascular Pharmacology, 3*(1), 63-68.

British National Formulary. (2014). Oral glucose tolerance test. *Diagnostic and monitoring devices in diabetes mellitus.* Retrieved from http://www.evidence.nhs.uk/formulary/bnf/current/6-endocrine-system/61-drugs-used-in-diabetes/616-diagnostic-and-monitoring-devices-for-diabetes-mellitus/oral-glucose-tolerance-test

Brown, A. J., & Roberts, D. C. K. (1991). The effect of postprandial fasting triacylglyceride concentration and apolipoprotein-E polymorphism on postprandial lipemia. *Arteriosclerosis and Thrombosis, 11*(6), 1737-1744.

Bucolo, G., & David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. *Clinical Chemistry, 19*(5), 476-482.

Burgomaster, K. A., Cermak, N. M., Phillips, S. M., Benton, C. R., Bonen, A., & Gibala, M. J. (2007). Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 292*(5), R1970-1976. doi:10.1152/ajpregu.00503.2006

Burgomaster, K. A., Heigenhauser, G. J., & Gibala, M. J. (2006). Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. *J Appl Physiol (1985), 100*(6), 2041-2047. doi:10.1152/japplphysiol.01220.2005

Burgomaster, K. A., Howarth, K. R., Phillips, S. M., Rakobowchuk, M., MacDonald, M. J., McGee, S. L., & Gibala, M. J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *The Journal of Physiology, 586*(1), 151-160. doi:10.1113/jphysiol.2007.142109

Burgomaster, K. A., Hughes, S. C., Heigenhauser, G. J., Bradwell, S. N., & Gibala, M. J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol (1985), 98*(6), 1985-1990. doi:10.1152/japplphysiol.01095.2004

Burns, S. F., Miyashita, M., & Stensel, D. J. (2015). High-Intensity Interval Exercise and Postprandial Triacylglycerol. *Sports Medicine, 45*(7), 957-968. doi:10.1007/s40279-015-0327-6

Carroll, M., Kit, B., & Lacher, D. (2015). Trends in elevated triglyceride in adults: United States, 2001-2012. *NCHS Data Brief*(198), 198.

Cassidy, S., Thoma, C., Houghton, D., & Trenell, M. I. (2017). High-intensity interval training: a review of its impact on glucose control and cardiometabolic health. *Diabetologia, 60*(1), 7-23. doi:10.1007/s00125-016-4106-1

Castro Cabezas, M., Halkes, C. J. M., Meijssen, S., van Oostrom, A. J. H. H. M., & Erkelens, D. W. (2001). Diurnal triglyceride profiles: a novel approach to study triglyceride changes. *Atherosclerosis, 155*(1), 219-228. doi:10.1016/S0021-9150(00)00554-2

Chandran, M., Phillips, S. A., Ciaraldi, T., & Henry, R. R. (2003). Adiponectin: More than just another fat cell hormone? *Diabetes Care, 26*(8), 2442-2450. doi:10.2337/diacare.26.8.2442

Chavanelle, V., Boisseau, N., Otero, Y. F., Combaret, L., Dardevet, D., Montaurier, C., . . . Sirvent, P. (2017). Effects of high-intensity interval training and moderate-intensity continuous training on glycaemic control and skeletal muscle mitochondrial function in db/db mice. *Scientific Reports, 7*. doi:10.1038/s41598-017-00276-8

Chen, X. P., & Yang, W. Y. (2015). Branched-chain amino acids and the association with type2 diabetes. *Journal of Diabetes Investigation, 6*(4), 369-370. doi:10.1111/jdi.12345

Cochran, A. J., Percival, M. E., Tricarico, S., Little, J. P., Cermak, N., Gillen, J. B., . . . Gibala, M. J. (2014). Intermittent and continuous high-intensity exercise training induce similar acute but different chronic muscle adaptations. *Experimental Physiology, 99*(5), 782-791. doi:10.1113/expphysiol.2013.077453

Cohn, J. S., Johnson, E. J., Millar, J. S., Cohn, S. D., Milne, R. W., Marcel, Y. L., . . . Schaefer, E. J. (1993). Contribution of apoB-48 and apoB-100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma

concentration of TRL triglycerides and retinyl esters. *Journal of Lipid Research, 34*(12), 2033-2040.

Cortes, V. A., Busso, D., Maiz, A., Arteaga, A., Nervi, F., & Rigotti, A. (2014). Physiological and pathological implications of cholesterol. *Front Biosci (Landmark Ed), 19*, 416-428.

Cox-York, K. A., Sharp, T. A., Stotz, S. A., Bessesen, D. H., Pagliassotti, M. J., & Horton, T. J. (2013). The effects of sex, metabolic syndrome and exercise on postprandial lipemia. *Metabolism, 62*(2), 244-254. doi:10.1016/j.metabol.2012.08.003

Crouse, S. F., Obrien, B. C., Grandjean, P. W., Lowe, R. C., Rohack, J. J., & Green, J. S. (1997). Effects of training and a single session of exercise on lipids and apolipoproteins in hypercholesterolemic men. *Journal of Applied Physiology, 83*(6), 2019-2028.

Dallongeville, J., Gruson, E., Dallinga-Thie, G., Pigeyre, M., Gomila, S., & Romon, M. (2007). Effect of weight loss on the postprandial response to high-fat and high-carbohydrate meals in obese women. *European Journal of Clinical Nutrition, 61*(6), 711-718. doi:10.1038/sj.ejcn.1602603

Davis, N. E. (2005). Atherosclerosis--an inflammatory process. *Journal of Insurance Medicine, 37*(1), 72-75.

De Backer, G., Ambrosioni, E., Borch-Johnsen, K., Brotons, C., Cifkova, R., Dallongeville, J., . . . Wood, D. (2003). European guidelines on cardiovascular disease and prevention in clinical practice. *Atherosclerosis, 171*(1), 145-155.

De Man, F. H., Cabezas, M. C., Van Barlingen, H. H., Erkelens, D. W., & de Bruin, T. W. (1996). Triglyceride-rich lipoproteins in non-insulin-dependent diabetes mellitus: post-prandial metabolism and relation to premature atherosclerosis. *European Journal of Clinical Investigation, 26*(2), 89-108.

de Souza, E. O., Tricoli, V., Aoki, M. S., Roschel, H., Brum, P. C., Bacurau, A. V. N., . . . Ugrinowitsch, C. (2014). Effects of concurrent strength and endurance training on genes related to myostatin signaling pathway and muscle fiber responses. *Journal of Strength and Conditioning Research, 28*(11), 3215-3223. doi:10.1519/jsc.0000000000000525

Despres, J. P., Moorjani, S., Lupien, P. J., Tremblay, A., Nadeau, A., & Bouchard, C. (1990). Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis, 10*(4), 497-511.

Drexel, H., Pfister, R., Mitterbauer, G., Föger, B. H., Lechleitner, M., Hörtnagl, H., & Patsch, J. R. (1992). Postprandial lipid and glucose metabolism in women undergoing moderate weight loss by diet plus exercise. . *Nutrition, Metabolism, and Cardiovascular Diseases, 2*, 159-164.

Dubois, C., Beaumier, G., Juhel, C., Armand, M., Portugal, H., Pauli, A. M., . . . Lairon, D. (1998). Effects of graded amounts (0-50 g) of dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. *American Journal of Clinical Nutrition, 67*(1), 31-38.

Dufaux, B., Order, U., Muller, R., & Hollmann, W. (1986). Delayed effects of prolonged exercise on serum lipoproteins. *Metabolism: Clinical and Experimental, 35*(2), 105-109.

Duncan, G. E., Perri, M. G., Theriaque, D. W., Hutson, A. D., Eckel, R. H., & Stacpoole, P. W. (2003). Exercise Training, Without Weight Loss, Increases Insulin Sensitivity and Postheparin Plasma Lipase Activity in Previously Sedentary Adults. *Diabetes Care, 26*(3), 557-562. doi:10.2337/diacare.26.3.557

Ericsson, M., Johnson, O., Tollin, C., Furberg, B., Backman, C., & Angquist, K. (1982). Serum lipoproteins, apolipoproteins and intravenous fat tolerance in young athletes. *Scandinavian Journal of Rehabilitation Medicine, 14*(4), 209-212.

Eskelinen, J.-J., Heinonen, I., Loyttyniemi, E., Saunavaara, V., Kirjavainen, A., Virtanen, K. A., . . . Kalliokoski, K. K. (2015). Muscle-specific glucose and free fatty acid uptake after sprint interval and moderate-intensity training in healthy middle-aged men. *Journal of Applied Physiology, 118*(9), 1172-1180. doi:10.1152/japplphysiol.01122.2014

Estes, R. R., Malinowski, A., Piacentini, M., Thrush, D., Salley, E., Losey, C., & Hayes, E. (2017). The Effect of High Intensity Interval Run Training on Cross-sectional Area of the Vastus Lateralis in Untrained College Students. *International journal of exercise science, 10*(1), 137-145.

Feingold, K. R., & Grunfeld, C. (2000). Introduction to Lipids and Lipoproteins. In L. J. De Groot, G. Chrousos, K. Dungan, K. R. Feingold, A. Grossman, J. M. Hershman, C. Koch, M. Korbonits, R. McLachlan, M. New, J. Purnell, R. Rebar, F. Singer, & A. Vinik (Eds.), *Endotext*. South Dartmouth (MA): MDText.com, Inc.

Ferreira, A. P., Ferreira, C. B., Brito, C. J., Souza, V. C., Cordova, C., Nobrega, O. T., & Franca, N. M. (2013). The effect of aerobic exercise intensity on attenuation of postprandial lipemia is dependent on apolipoprotein E genotype. *Atherosclerosis, 229*(1), 139-144. doi:10.1016/j.atherosclerosis.2013.03.027

Ferreira, A. P., Ferreira, C. B., de Souza, V. C., de Almeida Córdova, C. O., Silva, G. C. B., de Toledo Nóbrega, O., & de França, N. M. (2011). The influence of intense intermittent versus moderate continuous exercise on postprandial lipemia. *Clinics, 66*(4), 535-541. doi:10.1590/S1807-59322011000400003

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology, 55*(2), 628-634.

Frayn, K. N., Coppack, S. W., Fielding, B. A., & Humphreys, S. M. (1995). Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue in vivo: Implications for the control of fat storage and fat mobilization. *Advances in Enzyme Regulation, 35*, 163-178.

Freese, E. C., Gist, N. H., Acitelli, R. M., McConnell, W. J., Beck, C. D., Hausman, D. B., . . . Evans, E. M. (2015). Acute and chronic effects of sprint interval exercise on postprandial lipemia in women at-risk for the metabolic

syndrome. *J Appl Physiol (1985), 118*(7), 872-879. doi:10.1152/japplphysiol.00380.2014

Freese, E. C., Gist, N. H., & Cureton, K. J. (2014). Effect of prior exercise on postprandial lipemia: an updated quantitative review. *Journal of Applied Physiology, 116*(1), 67-75. doi:10.1152/japplphysiol.00623.2013

Freese, E. C., Levine, A. S., Chapman, D. P., Hausman, D. B., & Cureton, K. J. (2011). Effects of acute sprint interval cycling and energy replacement on postprandial lipemia. *Journal of Applied Physiology, 111*(6), 1584-1589. doi:10.1152/japplphysiol.00416.2011

Frick, G. P., & Goodman, H. M. (1989). Insulin regulation of the activity and phosphorylation of branched-chain 2-oxo acid dehydrogenase in adipose-tissue. *Biochemical Journal, 258*(1), 229-235.

Fujita, S., Rasmussen, B. B., Cadenas, J. G., Drummond, M. J., Glynn, E. L., Sattler, F. R., & Volpi, E. (2007). Aerobic exercise overcomes the age-related insulin resistance of muscle protein metabolism by improving endothelial function and Akt/mammalian target of rapamycin signaling. *Diabetes, 56*(6), 1615-1622. doi:10.2337/db06-1566

Fukuda, N., Tojho, M., Hidaka, T., Sho, H., & Sugano, M. (1991). Reciprocal responses to exercise in hepatic ketogenesis and lipid secretion in the rat. *Annals of Nutrition and Metabolism, 35*(4), 233-241.

Gabriel, B., Pugh, J., Pruneta-Deloche, V., Moulin, P., Ratkevicius, A., & Gray, S. R. (2013). The effect of high intensity interval exercise on postprandial triacylglycerol and leukocyte activation--monitored for 48 h post exercise. *PloS One, 8*(12), e82669. doi:10.1371/journal.pone.0082669

Gabriel, B., Ratkevicius, A., Gray, P., Frenneaux, M. P., & Gray, S. R. (2012). High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress. *Clinical Science (London, England: 1979), 123*(5), 313-321. doi:10.1042/cs20110600

Gaenzer, H., Neumayr, G., Marschang, P., Strum, W., Kirchmair, R., & Patsch, J. R. (2001). Flow-mediated vasodilation of the femoral and brachial artery induced by exercise in healthy nonsmoking and smoking men. *Journal of the American College of Cardiology, 38*(5), 1313-1319. doi:10.1016/s0735-1097(01)01575-3

Galkina, E., & Ley, K. (2009). Immune and inflammatory mechanisms of atherosclerosis (*). *Annual Review of Immunology, 27*, 165-197. doi:10.1146/annurev.immunol.021908.132620

Gardner, A. W., & Parker, D. E. (2010). Association between arterial compliance and age in participants 9 to 77 years old. *Angiology, 61*(1), 37-41. doi:10.1177/0003319709339588

George, S. J., & Johnson, J. (2010). *Atherosclerosis Molecular and Cellular Mechanisms*. Weinheim, Germany: Wiley-VCH.

Ghosh, S., Zhao, B., Bie, J., & Song, J. (2010). Macrophage cholesteryl ester mobilization and atherosclerosis. *Vascular Pharmacology, 52*(1-2), 1-10. doi:10.1016/j.vph.2009.10.002

Gill, J. M., Al-Mamari, A., Ferrell, W. R., Cleland, S. J., Perry, C. G., Sattar, N., . . . Petrie, J. R. (2007). Effect of prior moderate exercise on postprandial metabolism in men with type 2 diabetes: heterogeneity of responses. *Atherosclerosis, 194*(1), 134-143. doi:10.1016/j.atherosclerosis.2006.10.007

Gill, J. M., Frayn, K. N., Wootton, S. A., Miller, G. J., & Hardman, A. E. (2001). Effects of prior moderate exercise on exogenous and endogenous lipid metabolism and plasma factor VII activity. *Clinical Science (London, England: 1979), 100*(5), 517-527.

Gill, J. M., & Hardman, A. E. (2000). Postprandial lipemia: effects of exercise and restriction of energy intake compared. *American Journal of Clinical Nutrition, 71*(2), 465-471.

Gill, J. M., & Hardman, A. E. (2003). Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets (review). *The Journal of Nutritional Biochemistry, 14*(3), 122-132.

Gill, J. M., Herd, S. L., & Hardman, A. E. (2002). Moderate exercise and post-prandial metabolism: issues of dose-response. *Journal of Sports Sciences, 20*(12), 961-967. doi:10.1080/026404102321011715

Gill, J. M., Herd, S. L., Vora, V., & Hardman, A. E. (2003). Effects of a brisk walk on lipoprotein lipase activity and plasma triglyceride concentrations in the fasted and postprandial states. *European Journal of Applied Physiology, 89*(2), 184-190. doi:10.1007/s00421-002-0788-9

Gill, J. M., Mees, G. P., Frayn, K. N., & Hardman, A. E. (2001). Moderate exercise, postprandial lipaemia and triacylglycerol clearance. *European Journal of Clinical Investigation, 31*(3), 201-207. doi:10.1046/j.1365-2362.2001.00799.x

Gillen, J. B., Little, J. P., Punthakee, Z., Tarnopolsky, M. A., Riddell, M. C., & Gibala, M. J. (2012). Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes. *Diabetes Obes Metab, 14*(6), 575-577. doi:10.1111/j.1463-1326.2012.01564.x

Gillen, J. B., Martin, B. J., MacInnis, M. J., Skelly, L. E., Tarnopolsky, M. A., & Gibala, M. J. (2016). Twelve Weeks of Sprint Interval Training Improves Indices of Cardiometabolic Health Similar to Traditional Endurance Training despite a Five-Fold Lower Exercise Volume and Time Commitment. *PLoS One, 11*(4), e0154075. doi:10.1371/journal.pone.0154075

Golan, D. E., & Tashjian, A. H. (2012). *Principles of pharmacology : the pathophysiologic basis of drug therapy*. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Goldberg, I. J., Scheraldi, C. A., Yacoub, L. K., Saxena, U., & Bisgaier, C. L. (1990). Lipoprotein ApoC-II activation of lipoprotein lipase. Modulation by apolipoprotein A-IV. *Journal of Biological Chemistry, 265*(8), 4266-4272.

Goodpaster, B. H., He, J., Watkins, S., & Kelley, D. E. (2001). Skeletal muscle lipid content and insulin resistance: Evidence for a paradox in endurance-trained

athletes. *Journal of Clinical Endocrinology & Metabolism, 86*(12), 5755-5761. doi:10.1210/jc.86.12.5755

Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B., & Dawber, T. R. (1977). High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. *The American Journal of Medicine, 62*(5), 707-714. doi:10.1016/0002-9343(77)90874-9

Goris, A. H., Westerterp-Plantenga, M. S., & Westerterp, K. R. (2000). Undereating and underrecording of habitual food intake in obese men: selective underreporting of fat intake. *The American Journal of Clinical Nutrition, 71*(1), 130-134.

Gustafsson, M., & Boren, J. (2004). Mechanism of lipoprotein retention by the extracellular matrix. *Current Opinion in Lipidology, 15*(5), 505-514.

Gylling, H., & Simonen, P. (2015). Phytosterols, Phytostanols, and Lipoprotein Metabolism. *Nutrients, 7*(9), 7965-7977. doi:10.3390/nu7095374

Hardman, A. E., Lawrence, J. E. M., & Herd, S. L. (1998). Postprandial lipemia in endurance-trained people during a short interruption to training. *Journal of Applied Physiology, 84*(6), 1895-1901.

Havel, R. J. (1998). Receptor and non-receptor mediated uptake of chylomicron remnants by the liver. *Atherosclerosis, 141, Supplement 1*, S1-S7. doi:10.1016/S0021-9150(98)00211-1

Heding, L. G. (1972). Determination of total serum insulin (IRI) in insulin-treated diabetic patients. *Diabetologia, 8*(4), 260-266. doi:10.1007/bf01225569

Hegele, R. A., Ginsberg, H. N., Chapman, M. J., Nordestgaard, B. G., Kuivenhoven, J. A., Averna, M., . . . Wiklund, O. (2014). The polygenic nature of hypertriglyceridaemia: implications for definition, diagnosis, and management. *Lancet Diabetes Endocrinol, 2*(8), 655-666. doi:10.1016/s2213-8587(13)70191-8

Henson, J., Davies, M. J., Bodicoat, D. H., Edwardson, C. L., Gill, J. M. R., Stensel, D. J., . . . Yates, T. (2016). Breaking Up Prolonged Sitting With Standing or Walking Attenuates the Postprandial Metabolic Response in Postmenopausal Women: A Randomized Acute Study. *Diabetes Care, 39*(1), 130-138. doi:10.2337/dc15-1240

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

Herd, S. L., Kiens, B., Boobis, L. H., & Hardman, A. E. (2001). Moderate exercise, postprandial lipemia, and skeletal muscle lipoprotein lipase activity. *Metabolism: Clinical and Experimental, 50*(7), 756-762. doi:10.1053/meta.2001.24199

Herd, S. L., Lawrence, J. E. M., Malkova, D., Murphy, M. H., Mastana, S., & Hardman, A. E. (2000). Postprandial lipemia in young men and women of contrasting training status. *Journal of Applied Physiology, 89*(5), 2049-2056.

Heron-Milhavet, L., Haluzik, M., Yakar, S., Gavrilova, O., Pack, S., Jou, W. C., . . . LeRoith, D. (2004). Muscle-specific overexpression of CD36 reverses the

insulin resistance and diabetes of MKR mice. *Endocrinology, 145*(10), 4667-4676. doi:10.1210/en.2003-1543

Herrington, W., Lacey, B., Sherliker, P., Armitage, J., & Lewington, S. (2016). Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. *Circulation Research, 118*(4), 535-546. doi:10.1161/circresaha.115.307611

Hokanson, J. E., & Austin, M. A. (1996). Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *Journal of Cardiovascular Risk, 3*(2), 213-219.

Holloszy, J. O., & Booth, F. W. (1976). Biochemical Adaptations to Endurance Exercise in Muscle. *Annual Review of Physiology, 38*(1), 273-291. doi:doi:10.1146/annurev.ph.38.030176.001421

Hood, M. S., Little, J. P., Tarnopolsky, M. A., Myslik, F., & Gibala, M. J. (2011). Low-volume interval training improves muscle oxidative capacity in sedentary adults. *Medicine and Science in Sports and Exercise, 43*(10), 1849-1856. doi:10.1249/MSS.0b013e3182199834

Howarth, K. R., Burgomaster, K. A., Phillips, S. M., & Gibala, M. J. (2007). Exercise training increases branched-chain oxoacid dehydrogenase kinase content in human skeletal muscle. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 293*(3), R1335-R1341. doi:10.1152/ajpregu.00115.2007

Hurren, N. M., Balanos, G. M., & Blannin, A. K. (2011). Is the beneficial effect of prior exercise on postprandial lipaemia partly due to redistribution of blood flow? *Clin Sci (Lond), 120*(12), 537-548. doi:10.1042/cs20100460

Hussain, M. M., Kedees, M. H., Singh, K., Athar, H., & Jamali, N. Z. (2001). Signposts in the assembly of chylomicrons. *Frontiers in bioscience : a journal and virtual library, 6*, D320-331. Retrieved from doi:10.2741/hussain

Insull, W., Jr. (2009). The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. *American Journal of Medicine, 122*(1 Suppl), S3-s14. doi:10.1016/j.amjmed.2008.10.013

Jacobs, I., Esbjornsson, M., Sylven, C., Holm, I., & Jansson, E. (1987). Sprint training effects on muscle myoglobin, enzymes, fiber types, and blood lactate. *Medicine and Science in Sports and Exercise, 19*(4), 368-374.

James, A. P., Slivkoff-Clark, K., & Mamo, J. C. L. (2007). Prior exercise does not affect chylomicron particle number following a mixed meal of moderate fat content. *Lipids in Health and Disease, 6*. doi:10.1186/1476-511x-6-8

James, A. P., Watts, G. F., Barrett, P. H., Smith, D., Pal, S., Chan, D. C., & Mamo, J. C. (2003). Effect of weight loss on postprandial lipemia and low-density lipoprotein receptor binding in overweight men. *Metabolism: Clinical and Experimental, 52*(2), 136-141. doi:10.1053/meta.2003.50032

Jelleyman, C., Yates, T., O'Donovan, G., Gray, L. J., King, J. A., Khunti, K., & Davies, M. J. (2015). The effects of high-intensity interval training on

glucose regulation and insulin resistance: a meta-analysis. *Obesity Reviews, 16*(11), 942-961. doi:10.1111/obr.12317

Jousilahti, P., Vartiainen, E., Pekkanen, J., Tuomilehto, J., Sundvall, J., & Puska, P. (1998). Serum Cholesterol Distribution and Coronary Heart Disease Risk. *Observations and Predictions Among Middle-aged Population in Eastern Finland, 97*(11), 1087-1094. doi:10.1161/01.cir.97.11.1087

Kaizu, S., Kishimoto, H., Iwase, M., Fujii, H., Ohkuma, T., Ide, H., . . . Kitazono, T. (2014). Impact of Leisure-Time Physical Activity on Glycemic Control and Cardiovascular Risk Factors in Japanese Patients with Type 2 Diabetes Mellitus: The Fukuoka Diabetes Registry. *Plos One, 9*(6). doi:10.1371/journal.pone.0098768

Karstoft, K., Winding, K., Knudsen, S. H., James, N. G., Scheel, M. M., Olesen, J., . . . Solomon, T. P. J. (2014). Mechanisms behind the superior effects of interval vs continuous training on glycaemic control in individuals with type 2 diabetes: a randomised controlled trial. *Diabetologia, 57*(10), 2081-2093. doi:10.1007/s00125-014-3334-5

Karstoft, K., Winding, K., Knudsen, S. H., Nielsen, J. S., Thomsen, C., Pedersen, B. K., & Solomon, T. P. J. (2013). The Effects of Free-Living Interval Walking Training on Glycemic Control, Body Composition, and Physical Fitness in Type 2 Diabetic Patients A randomized, controlled trial. *Diabetes Care, 36*(2), 228-236. doi:10.2337/dc12-0658

Katsuda, S., & Kaji, T. (2003). Atherosclerosis and extracellular matrix. *J Atheroscler Thromb, 10*(5), 267-274.

Kelley, G. A., Kelley, K. S., & Vu Tran, Z. (2005). Aerobic exercise, lipids and lipoproteins in overweight and obese adults: a meta-analysis of randomized controlled trials. *International Journal of Obesity and Related Metabolic Disorders, 29*(8), 881-893.

Kiens, B., Lithell, H., Mikines, K. J., & Richter, E. A. (1989a). EFFECTS OF INSULIN AND EXERCISE ON MUSCLE LIPOPROTEIN-LIPASE ACTIVITY IN MAN AND ITS RELATION TO INSULIN ACTION. *Journal of Clinical Investigation, 84*(4), 1124-1129. doi:10.1172/jci114275

Kiens, B., Lithell, H., Mikines, K. J., & Richter, E. A. (1989b). Effects of insuling and exercise on muscle lipoprotein-lipase activity in man and its relation to insulin action. *Journal of Clinical Investigation, 84*(4), 1124-1129. doi:10.1172/jci114275

Kiens, B., & Richter, E. A. (1998). Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. *American Journal of Physiology-Endocrinology and Metabolism, 275*(2), E332-E337.

Kolifa, M., Petridou, A., & Mougios, V. (2004). Effect of prior exercise on lipemia after a meal of moderate fat content. *European Journal of Clinical Nutrition, 58*(10), 1327-1335.

Kolovou, G. D., Mikhailidis, D. P., Kovar, J., Lairon, D., Nordestgaard, B. G., Ooi, T. C., . . . Panotopoulos, G. (2011). Assessment and clinical relevance of non-fasting and postprandial triglycerides: an expert panel statement. *Current Vascular Pharmacology, 9*(3), 258-270.

Koranyi, L. I., Bourey, R. E., Slentz, C. A., Holloszy, J. O., & Permutt, M. A. (1991). Coordinate reduction of rat pancreatic-islet glucokinase and proinsulin messenger-RNA by exercise training. *Diabetes, 40*(3), 401-404. doi:10.2337/diabetes.40.3.401

Krssak, M., Petersen, K. F., Dresner, A., DiPietro, L., Vogel, S. M., Rothman, D. L., . . . Roden, M. (1999). Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a H-1 NMR spectroscopy study. *Diabetologia, 42*(1), 113-116. doi:10.1007/s001250051123

Kulbertus, H., & Lancellotti, P. (2012). [Atherosclerosis: a complex disease]. *Rev Med Liege, 67*(5-6), 273-278.

Lagrost, L., Gandjini, H., Athias, A., Guyard-Dangremont, V., Lallemant, C., & Gambert, P. (1993). Influence of plasma cholesteryl ester transfer activity on the LDL and HDL distribution profiles in normolipidemic subjects. *Arteriosclerosis and Thrombosis, 13*(6), 815-825.

Lairon, D., Lopez-Miranda, J., & Williams, C. (2007). Methodology for studying postprandial lipid metabolism. *European Journal of Clinical Nutrition, 61*(10), 1145-1161. doi:10.1038/sj.ejcn.1602749

Lakatta, E. G. (1993). Cardiovascular regulatory mechanisms in advanced age. *Physiological Reviews, 73*(2), 413-467.

Leray, C. (2014). *Lipids*. London, UNKNOWN: CRC Press.

Ligtenberg, P. C., Hoekstra, J. B., Bol, E., Zonderland, M. L., & Erkelens, D. W. (1997). Effects of physical training on metabolic control in elderly type 2 diabetes mellitus patients. *Clinical Science (London, England: 1979), 93*(2), 127-135.

Little, J. P., Gillen, J. B., Percival, M. E., Safdar, A., Tarnopolsky, M. A., Punthakee, Z., . . . Gibala, M. J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J Appl Physiol (1985), 111*(6), 1554-1560. doi:10.1152/japplphysiol.00921.2011

Little, J. P., Jung, M. E., Wright, A. E., Wright, W., & Manders, R. J. (2014). Effects of high-intensity interval exercise versus continuous moderate-intensity exercise on postprandial glycemic control assessed by continuous glucose monitoring in obese adults. *Applied Physiology, Nutrition, and Metabolism. Physiologie Appliquée, Nutrition et Métabolisme, 39*(7), 835-841. doi:10.1139/apnm-2013-0512

Little, J. P., Safdar, A., Wilkin, G. P., Tarnopolsky, M. A., & Gibala, M. J. (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *Journal of Physiology, 588*(Pt 6), 1011-1022. doi:10.1113/jphysiol.2009.181743

MacDougall, J. D., Hicks, A. L., MacDonald, J. R., McKelvie, R. S., Green, H. J., & Smith, K. M. (1998). Muscle performance and enzymatic adaptations to sprint interval training. *Journal of Applied Physiology, 84*(6), 2138-2142.

MacInnis, M. J., & Gibala, M. J. (2016). Physiological adaptations to interval training and the role of exercise intensity. *Journal of Physiology*. doi:10.1113/jp273196

Madsen, S. M., Thorup, A. C., Overgaard, K., & Jeppesen, P. B. (2015). High Intensity Interval Training Improves Glycaemic Control and Pancreatic beta Cell Function of Type 2 Diabetes Patients. *Plos One, 10*(8). doi:10.1371/journal.pone.0133286

Magkos, F., Patterson, B. W., Mohammed, B. S., & Mittendorfer, B. (2007). A single 1-h bout of evening exercise increases basal FFA flux without affecting VLDL-triglyceride and VLDL-apolipoprotein B-100 kinetics in untrained lean men. *American Journal of Physiology - Endocrinology And Metabolism, 292*(6), E1568-E1574. doi:10.1152/ajpendo.00636.2006

Magkos, F., Wright, D. C., Patterson, B. W., Mohammed, B. S., & Mittendorfer, B. (2006). Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men. *American Journal of Physiology - Endocrinology And Metabolism, 290*(2), E355-E362. doi:10.1152/ajpendo.00259.2005

Maki, K. C., McKenney, J. M., Farmer, M. V., Reeves, M. S., & Dicklin, M. R. (2009). Indices of insulin sensitivity and secretion from a standard liquid meal test in subjects with type 2 diabetes, impaired or normal fasting glucose. *Nutrition Journal, 8*(1), 22. doi:10.1186/1475-2891-8-22

Maki, K. C., Phillips-Eakley, A. K., & Smith, K. N. (2016). The Effects of Breakfast Consumption and Composition on Metabolic Wellness with a Focus on Carbohydrate Metabolism. *Advances in Nutrition, 7*(3), 613S-621S. doi:10.3945/an.115.010314

Manning, B. D. (2004). Balancing Akt with S6K: implications for both metabolic diseases and tumorigenesis. *Journal of Cell Biology, 167*(3), 399-403. doi:10.1083/jcb.200408161

Maraki, M., Aggelopoulou, N., Christodoulou, N., Anastasiou, C. A., Toutouza, M., Panagiotakos, D. B., . . . Sidossis, L. S. (2011). Lifestyle intervention leading to moderate weight loss normalizes postprandial triacylglycerolemia despite persisting obesity. *Obesity (Silver Spring), 19*(5), 968-976. doi:10.1038/oby.2010.218

Maraki, M., Aggelopoulou, N., Christodoulou, N., Katsarou, C., Anapliotis, P., Kavouras, S. A., . . . Sidossis, L. S. (2011). Validity of abbreviated oral fat tolerance tests for assessing postprandial lipemia. *Clinical Nutrition, 30*(6), 852-857. doi:10.1016/j.clnu.2011.05.003

Maraki, M., Christodoulou, N., Aggelopoulou, N., Magkos, F., Skenderi, K. P., Panagiotakos, D., . . . Sidossis, L. S. (2009). Exercise of low energy expenditure along with mild energy intake restriction acutely reduces fasting and postprandial triacylglycerolaemia in young women. *British Journal of Nutrition, 101*(3), 408-416. doi:10.1017/s0007114508012233

Maraki, M., & Sidossis, L. S. (2010). Effects of energy balance on postprandial triacylglycerol metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care, 13*(6), 608-617. doi:10.1097/MCO.0b013e32833f1aae

Maraki, M., & Sidossis, L. S. (2013). The Latest on the Effect of Prior Exercise on Postprandial Lipaemia. *Sports medicine (Auckland, N.Z.), 43*(6), 463-481. doi:10.1007/s40279-013-0046-9

Mead, J. R., Irvine, S. A., & Ramji, D. P. (2002). Lipoprotein lipase: structure, function, regulation, and role in disease. *Journal of Molecular Medicine (Berlin, Germany), 80*(12), 753-769. doi:10.1007/s00109-002-0384-9

Mendis, S., Puska, P., & Norrving, B. (2011). Global Atlas on Cardiovascular Disease Prevention and Control. *World Health Organization, Geneva*.

Merrill, J. R., Holly, R. G., Anderson, R. L., Rifai, N., King, M. E., & DeMeersman, R. (1989). Hyperlipemic response of young trained and untrained men after a high fat meal. *Arteriosclerosis, 9*(2), 217-223.

Mezzani, A. (2017). Cardiopulmonary Exercise Testing: Basics of Methodology and Measurements. *Ann Am Thorac Soc, 14*(Supplement_1), S3-S11. doi:10.1513/AnnalsATS.201612-997FR

Mikus, C. R., Oberlin, D. J., Libla, J. L., Taylor, A. M., Booth, F. W., & Thyfault, J. P. (2012). Lowering Physical Activity Impairs Glycemic Control in Healthy Volunteers. *Medicine and Science in Sports and Exercise, 44*(2), 225-231. doi:10.1249/MSS.0b013e31822ac0c0

Miller, G. J., & Miller, N. E. (1975). Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *The Lancet, 305*(7897), 16-19. doi:10.1016/S0140-6736(75)92376-4

Miller, K. W., & Small, D. M. (1983). Surface-to-core and interparticle equilibrium distributions of triglyceride-rich lipoprotein lipids. *Journal of Biological Chemistry, 258*(22), 13772-13784.

Miyachi, M., Tanaka, H., Yamamoto, K., Yoshioka, A., Takahashi, K., & Onodera, S. (2001). Effects of one-legged endurance training on femoral arterial and venous size in healthy humans. *Journal of Applied Physiology, 90*(6), 2439-2444.

Miyashita, M., Burns, S. F., & Stensel, D. J. (2006). Exercise and postprandial lipemia: effect of continuous compared with intermittent activity patterns. *Am J Clin Nutr, 83*(1), 24-29.

Miyashita, M., Burns, S. F., & Stensel, D. J. (2008). Accumulating short bouts of brisk walking reduces postprandial plasma triacylglycerol concentrations and resting blood pressure in healthy young men. *American Journal of Clinical Nutrition, 88*(5), 1225-1231. doi:10.3945/ajcn.2008.26493

Miyashita, M., Burns, S. F., & Stensel, D. J. (2013). An update on accumulating exercise and postprandial lipaemia: translating theory into practice. *Journal of preventive medicine and public health = Yebang Uihakhoe chi, 46 Suppl 1*, S3-11. doi:10.3961/jpmph.2013.46.S.S3

Miyashita, M., Park, J. H., Takahashi, M., Suzuki, K., Stensel, D., & Nakamura, Y. (2013). Postprandial Lipaemia: Effects of Sitting, Standing and Walking in Healthy Normolipidaemic Humans. *International Journal of Sports Medicine, 34*(1), 21-27. doi:10.1055/s-0032-1321897

Morton, R. E. (1999). Cholesteryl ester transfer protein and its plasma regulator: lipid transfer inhibitor protein. *Current Opinion in Lipidology, 10*(4), 321-327.

Motiani, K. K., Savolainen, A. M., Eskelinen, J.-J., Toivanen, J., Ishizu, T., Yli-Karjanmaa, M., . . . Hannukainen, J. C. (2017). Two weeks of moderate-intensity continuous training, but not high-intensity interval training, increases insulin-stimulated intestinal glucose uptake. *Journal of Applied Physiology, 122*(5), 1188-1197. doi:10.1152/japplphysiol.00431.2016

Mulder, C., Schouten, J. A., & Popp-Snijders, C. (1983). Determination of free fatty acids: a comparative study of the enzymatic versus the gas chromatographic and the colorimetric method. *Journal of Clinical Chemistry and Clinical Biochemistry, 21*(12), 823-827.

Nagai, Y., Metter, E. J., Earley, C. J., Kemper, M. K., Becker, L. C., Lakatta, E. G., & Fleg, J. L. (1998). Increased Carotid Artery Intimal-Medial Thickness in Asymptomatic Older Subjects With Exercise-Induced Myocardial Ischemia. *Circulation, 98*(15), 1504-1509. doi:10.1161/01.cir.98.15.1504

Newgard, C. B. (2012). Interplay between Lipids and Branched-Chain Amino Acids in Development of Insulin Resistance. *Cell Metabolism, 15*(5), 606-614. doi:10.1016/j.cmet.2012.01.024

Nordestgaard, B. G., Benn, M., Schnohr, P., & Tybjaerg-Hansen, A. (2007). Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA, 298*(3), 299-308. doi:10.1001/jama.298.3.299

Nordestgaard, B. G., Stender, S., & Kjeldsen, K. (1988). Reduced atherogenesis in cholesterol-fed diabetic rabbits. Giant lipoproteins do not enter the arterial wall. *Arteriosclerosis, 8*(4), 421-428.

Nordestgaard, B. G., & Varbo, A. (2014). Triglycerides and cardiovascular disease. *Lancet, 384*(9943), 626-635. doi:10.1016/s0140-6736(14)61177-6

Nordestgaard, B. G., & Zilversmit, D. B. (1988). Large lipoproteins are excluded from the arterial wall in diabetic cholesterol-fed rabbits. *Journal of Lipid Research, 29*(11), 1491-1500.

Nurnberger, J., Dammer, S., Philipp, T., Wenzel, R. R., & Schafers, R. F. (2003). Metabolic and haemodynamic effects of oral glucose loading in young healthy men carrying the 825T-allele of the G protein beta 3 subunit. *Cardiovascular Diabetology, 2*. doi:10.1186/1475-2840-2-7

Ohshima, T., Misono, H., & Soda, K. (1978). Determination of Branched-chain l-Amino Acids and Their Keto Analogs with Leucine Dehydrogenase. *Agricultural and Biological Chemistry, 42*(10), 1919-1922. doi:10.1080/00021369.1978.10863275

Palmefors, H., DuttaRoy, S., Rundqvist, B., & Borjesson, M. (2014). The effect of physical activity or exercise on key biomarkers in atherosclerosis--a systematic review. *Atherosclerosis, 235*(1), 150-161. doi:10.1016/j.atherosclerosis.2014.04.026

Peddie, M. C., Rehrer, N. J., & Perry, T. L. (2012). Physical activity and postprandial lipidemia: Are energy expenditure and lipoprotein lipase

activity the real modulators of the positive effect? *Progress in Lipid Research, 51*(1), 11-22. doi:10.1016/j.plipres.2011.11.002

Perez-Martinez, P., Alcala-Diaz, J. F., Kabagambe, E. K., Garcia-Rios, A., Tsai, M. Y., Delgado-Lista, J., . . . Lopez-Miranda, J. (2016). Assessment of postprandial triglycerides in clinical practice: Validation in a general population and coronary heart disease patients. *Journal of Clinical Lipidology, 10*(5), 1163-1171. doi:10.1016/j.jacl.2016.05.009

Plaisance, E. P., & Fisher, G. (2014). Exercise and Dietary-Mediated Reductions in Postprandial Lipemia. *Journal of Nutrition and Metabolism, 2014*, 902065. doi:10.1155/2014/902065

Plaisance, E. P., Mestek, M. L., Mahurin, A. J., Taylor, J. K., Moncada-Jimenez, J., & Grandjean, P. W. (2008). Postprandial triglyceride responses to aerobic exercise and extended-release niacin. *American Journal of Clinical Nutrition, 88*(1), 30-37.

Podl, T. R., Zmuda, J. M., Yurgalevitch, S. M., Fahrenbach, M. C., Bausserman, L. L., Terry, R. B., & Thompson, P. D. (1994). Lipoprotein lipase activity and plasma triglyceride clearance are elevated in endurance-trained women. *Metabolism: Clinical and Experimental, 43*(7), 808-813.

Potteiger, J. A., Kirk, E. P., Jacobsen, D. J., & Donnelly, J. E. (2008). Changes in resting metabolic rate and substrate oxidation after 16 months of exercise training in overweight adults. *International Journal of Sport Nutrition and Exercise Metabolism, 18*(1), 79-95.

Potter, J. F., Heseltine, D., Hartley, G., Matthews, J., Macdonald, I. A., & James, O. F. W. (1989). Effects of meal composition on the postprandial blood-pressure, catecholamine and insulin changes in elderly subjects. *Clinical Science, 77*(3), 265-272.

Raitakari, O. T., Lai, N., Griffiths, K., McCredie, R., Sullivan, D., & Celermajer, D. S. (2000). Enhanced peripheral vasodilation in humans after a fatty meal. *Journal of the American College of Cardiology, 36*(2), 417-422. doi:10.1016/s0735-1097(00)00758-0

Redgrave, T. G. (2004). Chylomicron metabolism. *Biochemical Society Transactions, 32*(Pt 1), 79-82. doi:10.1042/

Roberson, D. W., Astorino, T. A., Allen, R. P., Trost, E., & Jurancich, M. (2010). Effect of High Intensity Interval Training (HIIT) on Substrate Utilization. *Faseb Journal, 24*.

Robinson, E., Durrer, C., Simtchouk, S., Jung, M. E., Bourne, J. E., Voth, E., & Little, J. P. (2015). Short-term high-intensity interval and moderate-intensity continuous training reduce leukocyte TLR4 in inactive adults at elevated risk of type 2 diabetes. *Journal of Applied Physiology, 119*(5), 508-516. doi:10.1152/japplphysiol.00334.2015

Ronnestad, B. R., Hansen, E. A., & Raastad, T. (2012). High volume of endurance training impairs adaptations to 12 weeks of strength training in well-trained endurance athletes. *European Journal of Applied Physiology, 112*(4), 1457-1466. doi:10.1007/s00421-011-2112-z

Russell, A. P. (2004). Lipotoxicity: the obese and endurance-trained paradox. *International Journal of Obesity, 28*, S66-S71. doi:10.1038/sj.ijo.0802859

Sadur, C. N., & Eckel, R. H. (1982). Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique. *Journal of Clinical Investigation, 69*(5), 1119-1125.

Sady, S. P., Cullinane, E. M., Saritelli, A., Bernier, D., & Thompson, P. D. (1988). Elevated high-density lipoprotein cholesterol in endurance athletes is related to enhanced plasma triglyceride clearance. *Metabolism: Clinical and Experimental, 37*(6), 568-572.

Sandvei, M., Jeppesen, P. B., Stoen, L., Litleskare, S., Johansen, E., Stensrud, T., . . . Jensen, J. (2012). Sprint interval running increases insulin sensitivity in young healthy subjects. *Archives of Physiology and Biochemistry, 118*(3), 139-147. doi:10.3109/13813455.2012.677454

Sawyer, B. J., Tucker, W. J., Bhammar, D. M., Ryder, J. R., Sweazea, K. L., & Gaesser, G. A. (2016). Effects of high-intensity interval training and moderate-intensity continuous training on endothelial function and cardiometabolic risk markers in obese adults. *Journal of Applied Physiology, 121*(1), 279-288. doi:10.1152/japplphysiol.00024.2016

Schiffelers, S. L. H., Saris, W. H. M., & van Baak, M. A. (2001). The effect of an increased free fatty acid concentration on thermogenesis and substrate oxidation in obese and lean men. *International Journal of Obesity, 25*(1), 33-38. doi:10.1038/sj.ijo.0801528

Schubert, M. M., Clarke, H. E., Seay, R. F., & Spain, K. K. (2017). Impact of 4 weeks of interval training on resting metabolic rate, fitness, and health-related outcomes. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*, 1-9. doi:10.1139/apnm-2017-0268

Schwartz, R. S., Shuman, W. P., Larson, V., Cain, K. C., Fellingham, G. W., Beard, J. C., . . . Abrass, I. B. (1991). The effect of intensive endurance exercise training on body-fat distribution in young and older men. *Metabolism-Clinical and Experimental, 40*(5), 545-551. doi:10.1016/0026-0495(91)90239-s

Sedgwick, M. J., Morris, J. G., Nevill, M. E., & Barrett, L. A. (2015). Effect of repeated sprints on postprandial endothelial function and triacylglycerol concentrations in adolescent boys. *Journal of Sports Sciences, 33*(8), 806-816. doi:10.1080/02640414.2014.964749

Seip, R. L., Mair, K., Cole, T. G., & Semenkovich, C. F. (1997). Induction of human skeletal muscle lipoprotein lipase gene expression by short-term exercise is transient. *American Journal of Physiology, 272*(2 Pt 1), E255-261.

Shanely, R. A., Zwetsloot, K. A., Triplett, N. T., Meaney, M. P., Farris, G. E., & Nieman, D. C. (2014). Human skeletal muscle biopsy procedures using the modified Bergstrom technique. *J Vis Exp*(91), 51812. doi:10.3791/51812

Shin, Y., Park, S., & Choue, R. (2009). Comparison of time course changes in blood glucose, insulin and lipids between high carbohydrate and high fat

meals in healthy young women. *Nutrition Research and Practice, 3*(2), 128-133. doi:10.4162/nrp.2009.3.2.128

Sigal, R. J., Kenny, G. P., Wasserman, D. H., & Castaneda-Sceppa, C. (2004). Physical activity/exercise and type 2 diabetes. *Diabetes Care, 27*(10), 2518-2539. doi:10.2337/diacare.27.10.2518

Sipila, S., Elorinne, M., Alen, M., Suominen, H., & Kovanen, V. (1997). Effects of strength and endurance training on muscle fibre characteristics in elderly women. *Clinical Physiology, 17*(5), 459-474. doi:10.1046/j.1365-2281.1997.05050.x

Smith, M. E., & Morton, D. G. (2001). *The Digestive Sytem: Basic science and clinical conditions*. London, UK: Harcourt Publishers Ltd.

Smith-Ryan, A. E. (2015). Enjoyment of high-intensity interval training in an overweight/obese cohort: a short report. *Clinical Physiology and Functional Imaging*. doi:10.1111/cpf.12262

Sondergaard, E., Rahbek, I., Sørensen, L. P., Christiansen, J. S., Gormsen, L. C., Jensen, M. D., & Nielsen, S. (2011). Effects of exercise on VLDL-triglyceride oxidation and turnover. *American Journal of Physiology - Endocrinology And Metabolism, 300*(5), E939-E944. doi:10.1152/ajpendo.00031.2011

Stamler, J. (1978). DIetary and serum lipids in the multifactorial etiology of atherosclerosis. *Archives of Surgery, 113*(1), 21-25. doi:10.1001/archsurg.1978.01370130023004

Stensvold, D., Tjonna, A. E., Skaug, E.-A., Aspenes, S., Stolen, T., Wisloff, U., & Slordahl, S. A. (2010). Strength training versus aerobic interval training to modify risk factors of metabolic syndrome. *Journal of Applied Physiology, 108*(4), 804-810. doi:10.1152/japplphysiol.00996.2009

Stiegler, P., Sparks, S. A., & Cunliffe, A. (2008). Moderate exercise, postprandial energy expenditure, and substrate use in varying meals in lean and obese men. *International Journal of Sport Nutrition and Exercise Metabolism, 18*(1), 66-78.

Strong, J. P., Malcom, G. T., McMahan, C. A., Tracy, R. E., Newman, W. P., 3rd, Herderick, E. E., & Cornhill, J. F. (1999). Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. *JAMA, 281*(8), 727-735.

Sugawara, J., Murakami, H., Maeda, S., Kuno, S., & Matsuda, N. (2001). Change in post-exercise vagal reactivation with exercise training and detraining in young men. *European Journal of Applied Physiology, 85*(3-4), 259-263. doi:10.1007/s004210100443

Tan, M., Chan Moy Fat, R., Boutcher, Y. N., & Boutcher, S. H. (2014). Effect of high-intensity intermittent exercise on postprandial plasma triacylglycerol in sedentary young women. *International Journal of Sport Nutrition and Exercise Metabolism, 24*(1), 110-118. doi:10.1123/ijsnem.2013-0094

Tan, M. S., Mok, A., Yap, M. C., & Burns, S. F. (2013). Effect of sprint interval versus continuous cycling on postprandial lipaemia. *Journal of Sports Sciences, 31*(9), 989-995. doi:10.1080/02640414.2012.759661

Tedgui, A., & Mallat, Z. (2006). Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiological Reviews, 86*(2), 515-581. doi:10.1152/physrev.00024.2005

Teeman, C. S., Kurti, S. P., Cull, B. J., Emerson, S. R., Haub, M. D., & Rosenkranz, S. K. (2016). Postprandial lipemic and inflammatory responses to high-fat meals: a review of the roles of acute and chronic exercise. *Nutrition & Metabolism, 13*(1), 80. doi:10.1186/s12986-016-0142-6

Thackray, A. E., Barrett, L. A., & Tolfrey, K. (2013). Acute high-intensity interval running reduces postprandial lipemia in boys. *Medicine and Science in Sports and Exercise, 45*(7), 1277-1284. doi:10.1249/MSS.0b013e31828452c1

Thackray, A. E., Barrett, L. A., & Tolfrey, K. (2016). High-Intensity Running and Energy Restriction Reduce Postprandial Lipemia in Girls. *Medicine and Science in Sports and Exercise, 48*(3), 402-411. doi:10.1249/mss.0000000000000788

Thijssen, D. H. J., Maiorana, A. J., O'Driscoll, G., Cable, N. T., Hopman, M. T. E., & Green, D. J. (2010). Impact of inactivity and exercise on the vasculature in humans. *European Journal of Applied Physiology, 108*(5), 845-875. doi:10.1007/s00421-009-1260-x

Thompson, P. D., Cullinane, E., Henderson, L. O., & Herbert, P. N. (1980). Acute effects of prolonged exercise on serum lipids. *Metabolism: Clinical and Experimental, 29*(7), 662-665.

Tolfrey, K., Doggett, A., Boyd, C., Pinner, S., Sharples, A., & Barrett, L. (2008). Postprandial triacylglycerol in adolescent boys: a case for moderate exercise. *Medicine and Science in Sports and Exercise, 40*(6), 1049-1056. doi:10.1249/MSS.0b013e31816770fe

Tolfrey, K., Thackray, A. E., & Barrett, L. A. (2014). Acute exercise and postprandial lipemia in young people. *Pediatric Exercise Science, 26*(2), 127-137. doi:10.1123/pes.2013-0126

Tonkin, A. (2003). *Atherosclerosis and Heart Disease*. London, United Kingdom: Martin Dunitz.

Torres-Leal, F. L., Fonseca-Alaniz, M. H., Teodoro, G. F. R., de Capitani, M. D., Vianna, D., Pantaleao, L. C., . . . Tirapegui, J. (2011). Leucine supplementation improves adiponectin and total cholesterol concentrations despite the lack of changes in adiposity or glucose homeostasis in rats previously exposed to a high-fat diet. *Nutrition & Metabolism, 8*. doi:10.1186/1743-7075-8-62

Towler, M. C., & Hardie, D. G. (2007). AMP-activated protein kinase in metabolic control and insulin signaling. *Circulation Research, 100*(3), 328-341. doi:10.1161/01.res.0000256090.42690.05

Townsend, N., Wickramasinghe, K., Williams, J., Bhatnagar, P., & Rayner, M. (2015). Physical Activity Statistics 2015. *British Heart Foundation: London.*

Trejo-Gutierrez, J. F., & Fletcher, G. (2007). Impact of exercise on blood lipids and lipoproteins. *Journal of Clinical Lipidology, 1*(3), 175-181. doi:10.1016/j.jacl.2007.05.006

Trinder, P. (1969). Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology, 22*(2), 158-161.

Trombold, J. R., Christmas, K. M., Machin, D. R., Kim, I. Y., & Coyle, E. F. (2013). Acute high-intensity endurance exercise is more effective than moderate-intensity exercise for attenuation of postprandial triglyceride elevation. *J Appl Physiol (1985), 114*(6), 792-800. doi:10.1152/japplphysiol.01028.2012

Tsekouras, Y. E., Magkos, F., Kellas, Y., Basioukas, K. N., Kavouras, S. A., & Sidossis, L. S. (2008). High-intensity interval aerobic training reduces hepatic very low-density lipoprotein-triglyceride secretion rate in men. *American Journal of Physiology: Endocrinology and Metabolism, 295*(4), E851-858. doi:10.1152/ajpendo.90545.2008

Tsetsonis, N. V., & Hardman, A. E. (1996). Reduction in postprandial lipemia after walking: influence of exercise intensity. *Medicine and Science in Sports and Exercise, 28*(10), 1235-1242.

Tsetsonis, N. V., Hardman, A. E., & Mastana, S. S. (1997). Acute effects of exercise on postprandial lipemia: a comparative study in trained and untrained middle-aged women. *American Journal of Clinical Nutrition, 65*(2), 525-533.

Turcotte, L. P., & Fisher, J. S. (2008). Skeletal Muscle Insulin Resistance: Roles of Fatty Acid Metabolism and Exercise. *Physical Therapy, 88*(11), 1279-1296. doi:10.2522/ptj.20080018

Turpeinen, A. K., Takala, T. O., Nuutila, P., Axelin, T., Luotolahti, M., Haaparanta, M., . . . Knuuti, J. (1999). Impaired free fatty acid uptake in skeletal muscle but not in myocardium in patients with impaired glucose tolerance - Studies with PET and 14(R, S)- F-18 fluoro-6-thia-heptadecanoic acid. *Diabetes, 48*(6), 1245-1250. doi:10.2337/diabetes.48.6.1245

Tyldum, G. A., Schjerve, I. E., Tjonna, A. E., Kirkeby-Garstad, I., Stolen, T. O., Richardson, R. S., & Wisloff, U. (2009). Endothelial dysfunction induced by post-prandial lipemia: complete protection afforded by high-intensity aerobic interval exercise. *Journal of the American College of Cardiology, 53*(2), 200-206. doi:10.1016/j.jacc.2008.09.033

Vadiveloo, M., Scott, M., Quatromoni, P., Jacques, P., & Parekh, N. (2014). Trends in dietary fat and high-fat food intakes from 1991 to 2008 in the Framingham Heart Study participants. *British Journal of Nutrition, 111*(4), 724-734. doi:10.1017/s0007114513002924

Vaitkevicius, P. V., Fleg, J. L., Engel, J. H., O'Connor, F. C., Wright, J. G., Lakatta, L. E., . . . Lakatta, E. G. (1993). Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation, 88*(4), 1456-1462. doi:10.1161/01.cir.88.4.1456

van Wijk, J. P., Cabezas, M. C., Halkes, C. J., & Erkelens, D. W. (2001). Effects of different nutrient intakes on daytime triacylglycerolemia in healthy, normolipemic, free-living men. *The American Journal of Clinical Nutrition, 74*(2), 171-178.

Vogiatzis, I., Terzis, G., Nanas, S., Stratakos, G., Simoes, D. C. M., Georgiadou, O., . . . Roussos, C. (2005). Skeletal muscle adaptations to interval training in patients with advanced COPD. *Chest, 128*(6), 3838-3845. doi:10.1378/chest.128.6.3838

Vos, T., Barber, R. M., Bell, B., Bertozzi-Villa, A., Biryukov, S., Bolliger, I., . . . Murray, C. J. (2015). Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet, 386*(9995), 743-800. doi:10.1016/s0140-6736(15)60692-4

Wang, T. J., Larson, M. G., Vasan, R. S., Cheng, S., Rhee, E. P., McCabe, E., . . . Gerszten, R. E. (2011). Metabolite profiles and the risk of developing diabetes. *Nature Medicine, 17*(4), 448-U483. doi:10.1038/nm.2307

Weintraub, M. S., Rosen, Y., Otto, R., Eisenberg, S., & Breslow, J. L. (1989). Physical exercise conditioning in the absence of weight loss reduces fasting and postprandial triglyceride-rich lipoprotein levels. *Circulation, 79*(5), 1007-1014. doi:10.1161/01.cir.79.5.1007

Weir, J. B. D. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *Journal of Physiology-London, 109*(1-2), 1-9.

Weiss, E. P., Fields, D. A., Mittendorfer, B., Haverkort, M. A., & Klein, S. (2008). Reproducibility of postprandial lipemia tests and validity of an abbreviated 4-hour test. *Metabolism: Clinical and Experimental, 57*(10), 1479-1485. doi:10.1016/j.metabol.2008.05.020

Weston, K. L., Azevedo, L. B., Bock, S., Weston, M., George, K. P., & Batterham, A. M. (2016). Effect of Novel, School-Based High-Intensity Interval Training (HIT) on Cardiometabolic Health in Adolescents: Project FFAB (Fun Fast Activity Blasts) - An Exploratory Controlled Before-And-After Trial. *PloS One, 11*(8), e0159116. doi:10.1371/journal.pone.0159116

Westphal, S., Orth, M., Ambrosch, A., Osmundsen, K., & Luley, C. (2000). Postprandial chylomicrons and VLDLs in severe hypertriacylglycerolemia are lowered more effectively than are chylomicron remnants after treatment with n-3 fatty acids. *American Journal of Clinical Nutrition, 71*(4), 914-920.

Westphal, S., Taneva, E., Kastner, S., Martens-Lobenhoffer, J., Bode-Boger, S., Kropf, S., . . . Luley, C. (2006). Endothelial dysfunction induced by postprandial lipemia is neutralized by addition of proteins to the fatty meal. *Atherosclerosis, 185*(2), 313-319. doi:10.1016/j.atherosclerosis.2005.06.004

Whayne, T. F., Jr. (2009). High-density lipoprotein cholesterol: current perspective for clinicians. *Angiology, 60*(5), 644-649. doi:10.1177/0003319709331392

Whyte, L. J., Gill, J. M. R., & Cathcart, A. J. (2010). Effect of 2 weeks of sprint interval training on health-related outcomes in sedentary overweight/obese men. *Metabolism-Clinical and Experimental, 59*(10), 1421-1428. doi:10.1016/j.metabol.2010.01.002

Williams, J. K., Armstrong, M. L., & Heistad, D. D. (1988). Vasa vasorum in atherosclerotic coronary arteries: responses to vasoactive stimuli and regression of atherosclerosis. *Circulation Research, 62*(3), 515-523.

Yoon, M.-S. (2016). The Emerging Role of Branched-Chain Amino Acids in Insulin Resistance and Metabolism. *Nutrients, 8*(7). doi:10.3390/nu8070405

Zhang, Y., Wei, D. D., Yuan, R. R., Ge, Q. X., Chen, F., Yang, S. X., . . . Shi, J. (2017). Effects of ApoE gene polymorphism on the efficacy of atorvastatin in the treatment of hyperlipidemia. *Zhonghua yi xue za zhi, 97*(4), 291-294. doi:10.3760/cma.j.issn.0376-2491.2017.04.011

Zimmermann, R., Strauss, J. G., Haemmerle, G., Schoiswohl, G., Birner-Gruenberger, R., Riederer, M., . . . Zechner, R. (2004). Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science, 306*(5700), 1383-1386. doi:10.1126/science.1100747