INTRODUCTION OF A NOVEL HOME-BASED HIGH-INTENSITY INTERVAL TRAINING PROGRAMME TO IMPROVE CARDIO-METABOLIC HEALTH IN AT-RISK INDIVIDUALS

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ABSTRACT

New strategies are urgently needed to increase physical activity participation in the increasingly sedentary population to combat the rising rates of obesity and metabolic disease. The aim of this thesis was to provide evidence that practical high-intensity interval training (HIT) strategies can remove many of the major exercise barriers for obese individuals and people with type 1 diabetes that could potentially increase physical activity participation. Secondly, this thesis aimed to provide mechanistic evidence to explain the physiological effectiveness of HIT as a means to reduce the risk of cardio-metabolic disease.

In **Chapters 4** and **5**, 32 obese adults with at least 3 additional cardiovascular disease (CVD) risk factors completed one of three 12-week training programmes 3x/week: Home-HIT (n=9); Laboratory-based supervised HIT (Lab-HIT; n=10) or home-based moderate intensity continuous training (Home-MICT; n=13). Changes in $\dot{V}O_{2peak}$, insulin sensitivity, body composition, flow-mediated dilation (FMD) and aortic pulse wave velocity (PWV) were assessed. Muscle biopsies were taken to assess changes in capillarisation, mitochondrial density, intramuscular triglyceride (IMTG) content and eNOS and GLUT4 protein expression using quantitative immunofluorescence microscopy. Adherence and compliance (Home-HIT 96±3% & 99±1%; Home-MICT 88±4% & 100±0%; Lab-HIT 97±1% & 100±0%, respectively) to training did not differ between groups. Training increased $\dot{V}O_{2peak}$ and Matsuda insulin sensitivity index (P<0.05). BMI, body fat percentage and visceral fat decreased (P<0.05). FMD increased and aortic PWV decreased in each group (P<0.05). Immunofluorescence microscopy revealed increased capillarisation, mitochondrial density, IMTG content and eNOS and GLUT4 protein expression (P<0.05).

In **Chapter 6**, 14 people with type 1 diabetes completed a randomised counterbalanced crossover design whereby continuous glucose monitoring was used to assess glycaemic control and risk of hypoglycaemia following a single bout of HIT and moderate-intensity continuous training (MICT) on separate days, compared to a non-exercise control day (CON). In **Chapter 7**, 14 people with type 1 diabetes (n=7 per group) completed six weeks of HIT or MICT 3x/week and the effect on glucose control and markers of cardio-metabolic health were measured. **Chapter 6** showed no difference in the time, incidence or severity of hypoglycaemia over the 24-hour or nocturnal period between the CON, HIT and MICT days. In

Chapter 7, six weeks of HIT or MICT improved $\dot{V}O_{2max}$ by 14% and 15%, respectively and aortic PWV by 12%, with no difference between groups. Therefore, **Chapters 6** and **7** demonstrate that HIT is an effective exercise strategy for people with type 1 diabetes that reduces the two major barriers of lack of time and fear of hypoglycaemia.

Finally, in **Chapter 8,** eleven previously sedentary individuals with type 1 diabetes completed 6 weeks of Home-HIT. Blood glucose was monitored before, immediately and 1h after all of the exercise sessions. Perceptions of the program along with attitudes towards exercise, barriers to exercise and previous experiences of exercise were evaluated using an online survey. Training session adherence was 93±2%, with participants achieving their target HR in 99±1% of sessions. Blood glucose was not different from baseline immediately or 1h post HIT exercise. Training increased $\dot{V}O_{2peak}$ by 8% (P=0.015), but blood pressure was unchanged (P=0.445). The qualitative data showed that the Home-HIT programme was positively received with many benefits.

In conclusion, this thesis provides strong evidence that HIT can reduce major barriers to exercise and potentially increase exercise participation in these at-risk populations. Furthermore, Home-HIT was shown to be an effective strategy to improve a wide range of physiological markers indicative of improved cardio-metabolic health. Importantly, Home-HIT not only reduced traditional barriers to exercise, but also the key barrier in people with type 1 diabetes, fear of hypoglycaemia. As such, Home-HIT may represent an effective strategy to improve health in obese individuals with elevated CVD and people with type 1 diabetes by increasing exercise participation. Future research should investigate the effects of Home-HIT on a larger scale using larger cohorts and longer training periods using large-scale randomised controlled trials.

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During completion of this PhD at Liverpool John Moores University, data from this thesis resulted in the following abstracts and conference communications:

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ABREVIATIONS

ADA	American Diabetes Association
AICAR	5-aminoimidazole-4-caboxamide-1-β-D-ribonucleoside
AIT	aerobic interval training
AMPK	AMP-activated protein kinase
AUC	area under the curve
BMI	body mass index
CaM	calmodulin
CC	capillary contacts
CD	capillary density
CEU	contrast enhanced ultrasound
C/Fı	capillary-to-fibre ratio on an individual fibre basis
CFPE	capillary-fibre perimeter exchange index
CGMS	continuous glucose monitoring system
CHO	carbohydrate
CON	control day of no exercise
COX IV	cytochrome oxidase IV
CVD	cardiovascular disease
DAG	diacylglycerol
DBP	diastolic blood pressure
DCCT	Diabetes Control and Compilations Trial
DXA	dual-energy x-ray absorptiometry
eNOS	endothelial nitric oxide synthase
ERK	extracellular related kinase
ET-1	endothelin-1
EXTOD	exercising for type 1 diabetes
FA	fibre cross-sectional area
FFA	free fatty acid
FMD	flow-mediated dilation

GLUT4	glucose transporter 4
GSK-3	glycogen synthase kinase-3
GSV	GLUT4 storage vesicles
HIT	high-intensity interval training
Home-HIT	home-based high-intensity interval training
Home-MICT	home-based moderate-intensity continuous training
HR	heart rate
HR_{max}	heart rate maximum
HSL	hormone sensitive lipase
IDDM	insulin-dependent diabetes mellitus
IKKβ	inhibitory kB kinase
IMTG	intramuscular triglyceride
IRS	insulin receptor substrate
ISI	insulin sensitivity index
JNK	c-Jun N-terminal kinase
Lab-HIT	laboratory-based high-intensity interval training
LCFA CoA	long-chain fatty acyl-CoA
LJMU	Liverpool John Moores University
LPL	lipoprotein lipase
MAP	mean arterial pressure
MAPK	mitogen-activated protein kinase
MICT	moderate-intensity continuous training
MRS	magnetic resonance spectroscopy
NAD(P)Hox	NAD(P)Hoxidase
NF-κB	nuclear factor-κB
NOX2	subunit of the NAD(P)Hox complex
NO	nitric oxide
O_2^-	superoxide
OGTT	oral glucose tolerance test

PDK-1	phosphoinositide-dependent kinase-1
PKB	protein kinase B
PKC	protein kinase C
PI3K	phosphatidylinositol 3-kinase
PIP_2	phosphatidylinositol 4,5-biphosphate
PIP_3	phosphatidylinositol 3,4,5-triphosphate
PWV	pulse wave velocity
ROS	reactive oxygen species
SBP	systolic blood pressure
Ser^{1177}	$serine^{1177}$
SH2	Src homology 2
SIT	sprint interval training
SMA	smooth muscle actin
SNARE	soluble N-ethylmaleimide sensitive factor attachment protein receptor
TAG	triacylglycerol
TG	triglyceride
UEA-I FITC	*Ulex europaeus*-FITC conjugated
$\dot{V}O_{2max}$	aerobic capacity
$\dot{V}O_{2peak}$	peak oxygen consumption
VSMC	vascular smooth muscle cells
WGA-350	wheat germ agglutinin-350
W_{max}	maximal power output

Chapter 1 Aims and Objectives

Chapter 1

The overarching aim of the thesis is to investigate the cardio-metabolic benefits of a practical form of high-intensity interval training (HIT), home-based HIT (Home-HIT), in obese individuals with elevated cardiovascular disease (CVD) risk and people with type 1 diabetes. Secondly, this thesis aims to provide clear mechanistic evidence to explain the physiological effectiveness of home-based HIT as a means to reduce the risk of cardio-metabolic disease. It is envisioned that this thesis will provide the first evidence for a practical exercise strategy that reduces the major barriers to exercise in these at-risk populations.

1.1. Objectives

These aims will be achieved through completion of five studies with the following objectives:

Study 1:

1. To investigate the adherence and compliance to a novel 12-week Home-HIT programme in obese individuals with elevated CVD risk.
2. To test whether Home-HIT improves markers of cardio-metabolic health including $\dot{V}O_{2peak}$, insulin sensitivity, blood lipid profile, arterial stiffness and body fat percentage.
3. To use immunofluorescence microscopy to assess changes in GLUT4, mitochondrial density and intramuscular triglycerides following 12 weeks of Home-HIT.

Study 2:

1. To investigate the effect of a 12-week Home-HIT intervention on skeletal muscle capillary density and skeletal muscle microvascular enzymes

responsible for nitric oxide (NO) production and NO quenching in previously sedentary obese individuals with elevated CVD risk.

Study 3:

1. To examine the effects of a single bout of HIT and moderate-intensity continuous training (MICT) following an overnight fast on acute and 24 hour glucose concentrations in people with type 1 diabetes compared to a day without exercise.
2. To assess the change in blood glucose concentrations during HIT and MICT in the fasted state using capillary blood sampling.

Study 4:

1. To investigate whether six weeks of HIT leads to similar improvements in cardio-metabolic health markers as MICT in people with type 1 diabetes.
2. To investigate the acute change in blood glucose concentration during HIT and MICT in the fed state.

Study 5:

1. To investigate the adherence and compliance of Home-HIT in people with type 1 diabetes.
2. To assess the effects of Home-HIT on $\dot{V}O_{2peak}$, insulin dose and glycaemic profile in people with type 1 diabetes.
3. To assess the acute effects of Home-HIT on blood glucose concentration in people with type 1 diabetes.
4. To use a qualitative survey to gain a greater understanding of the barriers to exercise that people with type 1 diabetes face and the participant experiences of Home-HIT.

1.2. Hypotheses

The hypotheses for each study are as follows:

Study 1:

1. Home-HIT will have high adherence at the prescribed exercise intensity (compliance).
2. Home-HIT will lead to comparable improvements in markers of cardio-metabolic health and traditional skeletal muscle training markers to the fully supervised laboratory-based HIT group.

Study 2:

1. Microvascular density and eNOS content will increase with Home-HIT alongside an increase in $\dot{V}O_{2peak}$ and insulin sensitivity.
2. Home-HIT will reduce the protein content of NOX2 and its activator $p47^{phox}$ in the endothelial layer of the terminal arterioles and capillaries.

Study 3:

1. Blood glucose concentration would be maintained following HIT and that the incidence and time spent in hypoglycaemia would be lower compared to MICT over the 24-hour period.

Study 4:

1. Six weeks of HIT will improve markers of cardio-metabolic health including $\dot{V}O_{2peak}$, glycaemic control, blood lipid profile and vascular health in people with type 1 diabetes to a similar degree to MICT.

Study 5:

1. Home-HIT will have high adherence and compliance in people with type 1 diabetes.
2. Six weeks of Home-HIT would increase $\dot{V}O_{2peak}$, reduce insulin requirements and improve glycaemic profile in people with type 1 diabetes.
3. Blood glucose concentration would remain stable up to 1 hour post Home-HIT sessions.

Chapter 2 General Introduction

Chapter 2

2.1. Introduction

As early as ~600 BC, the ancient Indian physician Susruta advocated regular exercise to minimise the consequences of obesity and diabetes (Tipton, 2008), stating that medical practice should direct as much effort to prevention of disease as to curative remedies (Bhishagratna, 1963). In the 20th Century, Morris et al. (1953) observed in their landmark study that incidence of coronary heart disease in sedentary bus drivers was double that of the physically active conductors in London double-decker buses, suggesting a protective effect of physical activity. Since then, the association between physical inactivity and chronic diseases has been acknowledged by countless mechanistic and epidemiological studies and in their excellent review, Booth et al. (2017) highlight how physical inactivity directly increases the risk of disease leading to reduced quality of life and increased mortality risk.

According to Neel's (1962) 'Thrifty Gene Hypothesis', the human genome adapted to efficiently store and utilise food to optimise metabolism to support the physically active lifestyle of our hunter-gatherer ancestors, some 10,000 years ago (Eaton et al., 1988). Because the genetic makeup of *Homo sapiens* has hardly changed since the Late-Palaeolithic era (50,000–10,000 BC), the sedentary, food-abundant state of the 21st Century is creating an abnormal phenotype which is associated with the increasing incidence of metabolic disease (Booth et al., 2012, Booth et al., 2017). Regular physical activity and/or aerobic training help preserve the normal, healthy human phenotype (Booth et al., 2017). Therefore, as suggested by Booth and Lees (2006), throughout this thesis the physically active, endurance trained phenotype will be referred to as the 'normal' or optimal state.

Changing eating habits towards a diet comprising high fat and sugar convenience foods with low nutritional value combined with an increasingly sedentary lifestyle has led to a huge increase in the prevalence of obesity and type

2 diabetes. There are ~350 million cases of type 2 diabetes worldwide which is projected to increase to ~500 million by 2035 (IDF Diabetes Atlas 8th Edition, 2017). This is associated with a huge financial burden, as 12% of global health expenditure is spent on diabetes treatment, equating to £523 billion per annum (IDF Diabetes Atlas 8th Edition, 2017). Physical inactivity has been identified as one of the leading global risks for mortality, and obesity has been shown to double the risk of all-cause mortality due to its association with cardio-metabolic pathologies such as cardiovascular disease (CVD) and type 2 diabetes (Berrington de Gonzalez et al., 2010, Kohl et al., 2012). However, the role of physical activity as a means to combat obesity and metabolic disease continues to be undervalued (Kohl et al., 2012). Low physical activity levels in the general population are largely due a number of exercise barriers, with the most common being a 'lack of time' (discussed in Section 2.6 and throughout this thesis). Major culture shifts leading to increased physical activity and/or novel strategies to reduce the barriers to exercise are urgently needed to combat the obesity crisis.

Type 1 diabetes is a chronic inflammatory autoimmune disease whereby the insulin-producing β-cells of the islets of Langerhans of the pancreas are destroyed (Atkinson and Eisenbarth, 2001). This leads to absolute insulin deficiency, making it a daily challenge to maintain euglycaemia (blood glucose 4-8 mmol/L) through self-administration of exogenous insulin, regular glucose monitoring and diet control. As with the rest of the population, people with type 1 diabetes are recommended to exercise regularly to reduce the risk of micro- and macrovascular complications (Vestberg et al., 2013, Price et al., 2014). However, people with type 1 diabetes tend to be at least as inactive as the general population with a large proportion of patients not maintaining a healthy body mass as ~60% are overweight or obese (Makura et al., 2013, Bohn et al., 2015). In addition to the usual barriers to exercise cited by the

general population there are further barriers to exercise for people with type 1 diabetes (Section 2.6.1.) that can reduce exercise participation.

This introduction will start by outlining how glucose control is achieved in the healthy, physically active state before introducing how inactivity and obesity lead to an unhealthy phenotype and impaired glucose homeostasis. The following sections will describe how exercise training can be used to combat insulin resistance and metabolic disease to provide context for the physiological adaptations investigated during the experimental chapters. The introduction will continue by discussing barriers to exercise faced by the general population and people with type 1 diabetes before describing the exercise strategies that have been employed in the literature to combat physical inactivity and metabolic disease.

2.2. Glucose Homeostasis

In normal healthy individuals, tight control mechanisms exist so that glucose uptake into peripheral tissues is precisely matched by the rate of endogenous glucose production to maintain plasma glucose concentration between 3.9-5.5 mmol/L (Abdul-Ghani et al., 2006, Gagliardino, 2005). Glucose is the primary fuel source of the brain, so severe reductions in blood glucose concentration (<2 mmol/L) are deleterious to brain function (Wasserman, 2009). On the other hand, prolonged and/or recurrent increases in blood glucose concentration (hyperglycaemia) leads to tissue damage and secondary complications including CVD, microvascular damage, retinopathy and nephropathy.

Blood glucose control is achieved by a number of hormones, of which insulin is possibly the most important and will be a large focus of this thesis. Insulin is a polypeptide hormone produced by the pancreatic β cells following a meal that regulates glucose homeostasis by promoting absorption of glucose from the blood

into skeletal muscle, liver and adipose tissue. In these tissues, the absorbed glucose is then either converted to glycogen via glycogenesis; converted to fatty acids through de novo lipogenesis; used to generate the glycerol backbone for triglycerides in adipose tissue; or used to provide energy through glycolysis. In the postabsorptive state, decreases in blood glucose concentration are sensed by α cells of the pancreas which secrete glucagon (Abdul-Ghani, 2006). Glucagon stimulates endogenous glucose output from the liver to maintain blood glucose concentration sufficient for normal brain function.

Skeletal muscle accounts for ~80% of glucose disposal during a hyperinsulinaemic-euglycaemic clamp (DeFronzo et al., 1981) and ~50% glucose disposal following ingestion of a mixed meal, with the splanchnic tissues accounting for the remaining 50% (Capaldo et al., 1999). Therefore, skeletal muscle glucose uptake is fundamental to the maintenance of glucose homeostasis following a meal or during exercise. Insulin-stimulated glucose uptake into skeletal muscle occurs primarily through facilitated diffusion via glucose transporters, of which glucose transporter 4 (GLUT4) is the predominant insulin-responsive isoform in human skeletal muscle (Watson and Pessin, 2001), although transendothelial glucose transport in skeletal muscle capillaries is mediated by GLUT1 (Wagenmakers et al., 2016). Insulin also stimulates increased perfusion of the microvascular system to promote greater delivery of insulin and glucose to the myocyte. Therefore, a high level of insulin sensitivity is required for an optimal healthy phenotype to prevent substantial perturbations in blood glucose concentrations and the long-term complications that can accompany a persistent elevation in blood glucose concentration (Abdul-Ghani et al., 2006). The following sections will describe the effects of insulin on microvascular perfusion and skeletal muscle glucose uptake in normal healthy individuals to provide context for future sections that discuss obesity-induced insulin resistance and type 1 diabetes.

2.2.1. Vascular Actions of Insulin

Insulin has important vascular actions that act to increase delivery of glucose and insulin to the myocyte (see Muniyappa et al. (2007) and Younk et al. (2014) for detailed reviews). Baron et al. (1990) introduced the idea that insulin could regulate its own delivery and that of glucose after experiments in which they observed a strong correlation between leg blood flow and insulin-mediated glucose disposal (Laakso et al., 1990, Baron et al., 1990). In the study by Baron et al. (1990) they measured whole body glucose uptake, leg glucose uptake (using the leg balance technique), leg blood flow and cardiac index after an overnight fast and over 180 minutes after an oral glucose load in lean and obese participants. In the lean group there was a 36% increase in peak leg blood flow over baseline while there was no change in the obese subjects. Consequently, leg glucose uptake was 44% lower in the obese than the lean group, suggesting that haemodynamics play an important role in glucose disposal and defects in blood flow response potentially contribute to decreased glucose tolerance and insulin resistance.

Studies using contrast enhanced ultrasound have shown that in the normal, healthy state microvascular blood volume increases within 15-30 minutes during a hyperinsulinaemic-euglycaemic clamp and that this correlates with the rise in forearm glucose uptake (Bonner et al., 2013, Eggleston et al., 2013, Eggleston et al., 2007). This is followed by relaxation of larger resistance vessels with increased overall limb blood flow after 90 minutes (Baron et al., 1996). Thus, insulin promotes its own delivery and the delivery of glucose to the skeletal muscle through nitric oxide (NO)-mediated dilation of the terminal arterioles leading to an increase in perfusion and expansion of the endothelial surface area to augment glucose uptake (Clerk et al., 2006, Vincent et al., 2004). Studies have shown that this response is related to insulin sensitivity, as obesity (Laakso et al., 1990), type 1 diabetes (Baron et al., 1991) and type 2 diabetes (Clark, 2008) have been shown to suppress insulin-mediated blood flow and glucose disposal. The postprandial increases in skeletal

muscle microvascular perfusion are important for glucose and lipid homeostasis, and maintenance of long-term metabolic health.

2.2.2. Mechanisms of Insulin on the Microvasculature

Insulin directly influences vascular function through increases of NO production and endothelin-1 (ET-1) synthesis within the endothelium to induce vasodilation and vasoconstriction, respectively (Muniyappa et al., 2007). Under insulin-sensitive conditions, NO is the predominant factor resulting in the vasodilatory response to an increase in insulin (Steinberg et al., 1994). Increases in plasma insulin concentration following a meal activates an insulin signalling cascade that shares many of the steps of the myocyte insulin signalling pathway (Section 2.2.3.), suggesting a common function and link between these actions of insulin.

Upon binding to insulin receptors on the vascular endothelium, insulin activates the IRS-1/PI3K/PDK-1/Akt signalling cascade (Montagnani et al., 2002b, Montagnani et al., 2002a, Montagnani et al., 2001) (Figure 2.1A.). First, insulin phosphorylates intracellular substrates including insulin receptor substrate (IRS) family members and the Shc that serve as docking proteins for downstream signalling molecules (White, 2002). Tyrosine phosphorylation of IRS creates Src homology 2 (SH2)-domain binding motifs for SH2-domain containing effectors including phosphatidylinositol 3-kinase (PI3K) and Grb-2. PI3K is a heterodimer composed of a regulatory p85 subunit and a catalytic p110 subunit. When SH2-domains of p85 subunit bind to tyrosine-phosphorylated motifs on IRS-1 this activates the p110 catalytic subunit to generate phosphatidylinositol 3,4,5-triphosphate (PIP_3) from phosphatidylinositol 4,5-biphosphate (PIP_2). PIP_3 binds to the pleckstrin-homology domain in the 3-phosphoinositide-dependent protein kinase-1 (PDK-1) resulting in phosphorylation and activation to phosphorylate and activate Akt and atypical protein kinase C (PKC) (Vanhaesebroeck and Alessi,

2000). Akt activates endothelial nitric oxide synthase (eNOS) via phosphorylation of Ser^{1177} (Dimmeler et al., 1999) resulting in NO production through the conversion of L-arginine to NO and L-citrulline (Nathan and Xie, 1994).

NO is a potent vasodilator that acts on the smooth muscle cell layer of the terminal arterioles leading to vasodilation and increased perfusion of the skeletal muscle to increase the surface area for the transport of insulin and glucose (Vincent et al., 2003). NO diffuses into the vascular smooth muscle cells (VSMC) to activate guanylate cyclase, driving relaxation of the VSMCs through an increase in cGMP (Fleming and Busse, 2003). Insulin at high concentrations also stimulates expression of ET-1 which is a potent vasoconstrictor that opposes the vasodilator action of NO (Marasciulo et al., 2006). The balance between NO and ET-1 protein synthesis is an important determinant of insulin's ability to increase skeletal muscle perfusion after meal ingestion and during hyperinsulinaemic-euglycaemic clamps (Ergul, 2011).

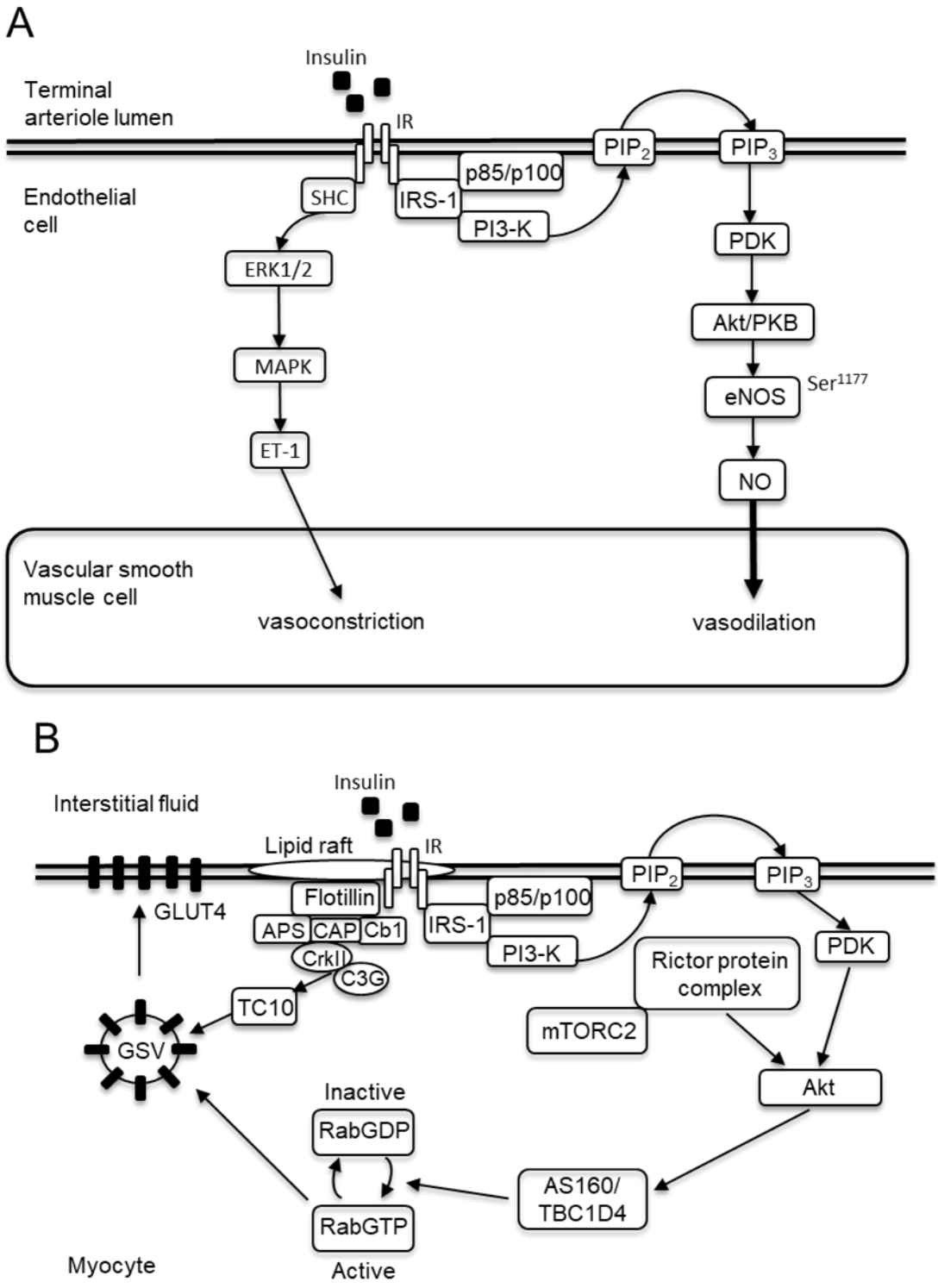

Figure 2.1. Insulin signalling cascades in the normal healthy state

A) Mechanisms of insulin on the microvasculature and B) myocyte in the normal insulin sensitive state. Adapted from Richter and Hargreaves (2013).

2.2.3. Insulin-Mediated Glucose Uptake into Skeletal Muscle

Following a postprandial rise in plasma glucose concentration, insulin is secreted from the pancreatic β cells into the circulation to promote glucose uptake into insulin sensitive tissues (Fridlyand and Phillipson, 2011). Insulin binds to the insulin receptor located on the skeletal muscle plasma membrane and initiates glucose uptake. The insulin receptor is a heterotetramer with two extracellular insulin binding α subunits and two membrane spanning β subunits (Chang et al., 2004). Insulin binds to the extracellular α subunit of the insulin receptor which stimulates tyrosine autophosphorylation of the insulin receptor β subunit and activation of tyrosine kinase (Chang et al., 2004). This promotes autophosphorylation and subsequent activation of IRS-1 to provide docking sites for downstream signalling proteins containing SH2-domains. Activation of IRS-1 triggers activation of PI3K and subsequent interaction with p110 catalytic and p85 regulatory subunits through increased interaction with SH2-domains (O'Neill, 2013). The activated p110 subunit of PI3K phosphorylates the 3'-OH moiety of the plasma membrane, PIP_2 to generate PIP_3. Increases in PIP_3 lead to activation of pleckstrin homology domain-containing proteins, such as PDK1, protein kinase B (Akt/PKB) and atypical PKC to localise these proteins to the plasma membrane (Chang et al., 2004). Akt is then phosphorylated by mammalian target of rapamycin complex 2 and Rictor protein complex on Ser^{473} and subsequently by PDK on Thr^{308} to achieve Akt activation (Sarbassov et al., 2005, Chang et al., 2004). Akt/PKB phosphorylates glycogen synthase kinase-3 (GSK-3), reducing the activity of this protein to subsequently increase the activity of glycogen synthase and thereby promote glycogen synthesis. Activated Akt/PKB also phosphorylates TBC1D4 (formally known as AS160). Under basal conditions, TBC1D4 retains Rab proteins in an inactive GDP-bound state thereby preventing GLUT4 translocation. In response to TBC1D4 phosphorylation, Rab proteins become GTP-loaded leading to a reorganisation of the cytoskeleton

that is required for GLUT4 mobilisation to the plasma membrane and glucose uptake.

Insulin-mediated GLUT4 translocation also occurs through an IRS-PI3K-independent pathway involving the APS-CAP-Cb1 complex. This pathway is centred on the lipid raft microdomain of the plasma membrane (see Figure 2.1B). The protein flotillin which is present in the lipid rafts interacts with CAP resulting in recruitment of the CAP/Cb1 so that it is incorporated within the lipid rafts (Chang et al., 2004). Tyrosine phosphorylation of Cb1 stimulates translocation of CrkII and C3G to the lipid rafts which activates the small G proteins TC10α and TC10β (Chiang et al., 2002). Upon activation, TC10 interacts with a number of effector molecules resulting in translocation of GLUT4.

In the basal state, GLUT4 slowly cycles from the plasma membrane to vesicle storage sites within the cell (Chang et al., 2004) and the majority of GLUT4 is located in intracellular storage clusters with a small proportion contained within the plasma membrane (Bryant and Gould, 2011). Following insulin stimulation, the slow cycling of GLUT4 between the plasma membrane and the intracellular storage membranes is interrupted and assumed to lead to increased GLUT4 at the plasma membrane through increased exocytosis of glucose storage vesicles and reduced endocytosis of GLUT4 (Kampmann et al., 2011, Richter and Hargreaves, 2013). During insulin-mediated glucose uptake, GLUT4 storage vesicles (GSV) containing 1-25 GLUT4 molecules are inserted into the muscle cell surface membrane following formation of specific SNARE (Soluble N-ethylmaleimide Sensitive Factor Attachment Protein Receptor) protein complexes.

Vesicle (v)-SNAREs are located in GLUT4 vesicles and target (t)-SNAREs are membrane proteins. A full overview of the role of SNAREs is beyond the focus of this introduction so readers are directed to reviews by Richter and Hargreaves (2013) and Bryant and Gould (2011). In brief, when v-SNAREs (e.g. VAMP2) interact with the relevant t-SNARE (e.g. SNAP23, syntaxin 4), a SNAREpin complex

is formed in which four SNARE motifs assemble to form a twisted parallel four helix bundle. This helical structure of the SNARE complex brings the lipid bilayer of the GLUT4 vesicle and plasma membrane into close proximity and then catalyses the fusion of vesicles so that GLUT4 can be incorporated within the plasma membrane. GLUT4 fusion is regulated through Munc18c which determines syntaxin 4 availability and SNARE complex formation. One model suggests that Munc18c is an inhibitory factor that under basal conditions is bound to syntaxin 4, which prevents VAMP2 from binding to form the SNARE complex (Tellam et al., 1997). Insulin stimulation activates aPKC resulting in phosphorylation of Munc18c. This reduces the Munc18c-syntaxin 4 interaction which allows the SNARE complex to assemble and then GSV fusion with the plasma membrane.

2.2.4. Contraction-Stimulated Glucose Uptake into Skeletal Muscle

Contraction-stimulated glucose uptake is preserved in people with insulin resistance and type 1 diabetes, emphasising the use of exercise as a therapeutic agent. This is because exercise-stimulated glucose transport does not rely on the proximal portion of the insulin signalling pathway (the insulin receptor, IRS and Akt) which is impaired with insulin resistance. An understanding of contraction-mediated glucose uptake is important for the purpose of this thesis because **Chapters 6** and **7** focus on glucose control in response to an acute bout of exercise in people with type 1 diabetes. Exercise increases glucose uptake up to 50-fold through simultaneous stimulation of delivery to the skeletal muscle through increased skeletal muscle perfusion, recruitment of additional capillary endothelial surface area leading to increased transendothelial transport of glucose via GLUT1 present in the luminal and abluminal endothelial membrane (Wagenmakers et al., 2016), increased glucose transport from the interstitial fluid surrounding the muscle fibres across the plasma membrane via GLUT4, and increased glycolysis and glucose oxidation in the muscle fibres. Studies using immuno-gold particle-labelling of GLUT4 combined with

electron microscopy have demonstrated that the effects of insulin and muscle contraction on GLUT4 redistribution are additive in rat skeletal muscle (Ploug et al., 1998). The additive nature of insulin and muscle contraction on GLUT4 translocation suggests there may be two intracellular pools of GLUT4: one recruited by insulin and the other by muscle contractions (Richter and Hargreaves, 2013). This is important to acknowledge when working with people with type 1 diabetes because exercise combined with increased circulating (exogenous) insulin will increase the risk of hypoglycaemia.

The molecular mechanisms leading to exercise-stimulated GLUT4 translocation are complex and no single master signalling pathway has been identified (Richter and Hargreaves, 2013). Nonetheless, it has been suggested that this regulation can be divided into a calcium (Ca^{2+})/calmodulin (CaM)-dependent feedforward mechanism and an AMP-activated protein kinase (AMPK)-related feedback mechanism responsible for adjusting glucose uptake of the muscle according to the muscle's energy needs. A thorough review of contraction-stimulated glucose uptake is beyond the scope of this introduction; therefore, interested readers are referred to the following reviews by Richter and Hargreaves (2013), Sakamoto and Goodyear (2002), and Sylow et al. (2017).

Evidence for a role of intracellular Ca^{2+} comes from early studies showing that Ca^{2+} is released from the sarcoplasmic reticulum of frog *sartorius* muscle incubated with caffeine which was associated with increased glucose transport (Holloszy and Narahara, 1965). Ca^{2+} release during muscle contractions is thought to increase glucose uptake via activation of other Ca^{2+}-sensitive downstream signalling molecules including CaMKK, CaMKII and nPKC (Rose and Richter, 2005, Rose et al., 2006, Jensen et al., 2014a). CaMKII has been implicated in contraction-induced glucose uptake because Jensen et al. (2007b) showed that unselective CaMKII blockers KN62 and KN93 decrease contraction-induced glucose uptake. Furthermore, inactivation of CaMK reduces glucose uptake in rat skeletal muscle

(Wright et al., 2005). However, it has since been shown that incubation with caffeine increases AMPK activation in mouse muscle, suggesting the increased cytosolic Ca^{2+} concentration in muscle is due to the increased energy demand of ion pumping even if the muscle is not contracting (Raney and Turcotte, 2008). It may therefore be that increased Ca^{2+} per se does not increase muscle glucose uptake but rather, the release of Ca^{2+} during muscle contractions and activation of the SERCA pump causes metabolic stress to muscle cells, and this stress by activation of AMPK causes an increase in muscle glucose uptake (Jensen et al., 2007a). This suggests an indirect effect of Ca^{2+} on muscle glucose uptake (Richter and Hargreaves, 2013).

AMPK is a serine/threonine protein kinase that senses the energy status of the muscle cell based on the AMP:ATP and creatine:phosphocreatine ratios (Salt et al., 1998). Therefore, as muscle contractions lead to an increase in the AMP:ATP ratio this causes AMPK activation (Wojtaszewski et al, 2000). Pharmacological activation of AMPK through AMP analogue 5-aminoimidazole-4-caboxamide-1-β-D-ribonucleoside (AICAR) in resting muscle has been shown to increase muscle glucose transport independently of insulin (Jorgensen et al., 2006). However, studies investigating the role of AMPK in contraction-mediated glucose uptake via AMPK knockout have proved inconclusive, as Mu et al. (2001) found that 60% of contraction-induced glucose uptake is maintained when AMPK is knocked out, suggesting other factors independent of AMPK may also stimulate glucose uptake. Therefore, the role of AMPK in contraction-induced glucose uptake is currently controversial, suggesting other factors are important.

Mechanical stress is also a potent stimulus to increase glucose transport in muscle in addition to alterations in the intracellular metabolic milieu (Jensen et al., 2014b, Sylow et al., 2015). Contraction-stimulated glucose uptake has been shown to be reduced when tension development is inhibited *ex vivo* (Jensen et al., 2014b, Sylow et al., 2015). Jensen et al. (2014b) found that a combination of passive stretch and AICAR-induced stimulation of AMPK promoted an additive effect on

glucose transport during co-stimulation with insulin but not muscle contraction. This suggests that passive stretch and AMPK recruit GLUT4 from the same pool. A number of stretch-activated pathways might be involved in glucose uptake including the Rho family GTPase RAC1 (Sylow et al., 2017). Pharmacological inhibition and gene knockout of RAC1 reduced stretch-induced glucose transport by 30-40% (Sylow et al., 2015). Mechanical stretch can be sensed by a number of proteins at the plasma membrane including dystrophin-glycoprotein complex, ion channels, integrins and focal adhesions which associate with and activate RAC1 (Sylow et al., 2017).

Muscle contractions also activate a number of mitogen-activated protein kinase (MAPK) isoforms; for example, extracellular related kinase 1 and 2 (ERK1/2), p38 and c-Jun N-terminal kinase (JNK) (Sakamoto and Goodyear, 2002). p38 MAPK has been implicated in contraction-induced glucose uptake because the drug SB203580 (a p38 MAPK inhibitor) decreases glucose uptake in rat muscle (Somwar et al., 2000). However, as SB203580 directly binds to GLUT4 in adipocytes this may not provide conclusive evidence for a role of p38 MAPK (Richter and Hargreaves, 2013). Further evidence is provided by the fact that activation of p38 MAPK in resting muscle using the drug animysin increases glucose uptake in rat muscle (Geiger et al., 2005). However, at present we do not have a complete understanding of the mechanisms through which p38 MAPK would lead to GLUT4 translocation.

Convergence of insulin and contraction-induced signalling occurs downstream of Akt at TBC1D1 and TBC1D4 as AMPK and CAMKII have been shown to phosphorylate TBC1D4 (Richter and Hargreaves, 2013). Phosphorylation of TBC1D4 inhibits Rab-GAP function leading to GTP loading and activation of target Rabs to promote GLUT4 translocation.

2.3. Lifestyle Factors Leading to Impaired Glucose Homeostasis

The previous sections focused on glucose delivery and uptake into the skeletal muscle to maintain glucose homeostasis in the normal insulin-sensitive state. The following sections will outline how insulin resistance impairs these actions resulting in impaired glucose homeostasis and eventually type 2 diabetes. Obesity and sedentary behaviour lead to structural and functional impairments in the skeletal muscle microvasculature that are associated with endothelial function loss and development of metabolic disease (Wasserman et al., 2018). Insulin resistance is also a common feature of type 1 diabetes (Kaul et al., 2015), which is exacerbated by the rising rates of obesity and physical inactivity in this population (Polsky and Ellis, 2015).

The time course for the development of type 2 diabetes is relatively slow and the metabolic abnormalities are established long before overt diabetes develops (Abdul-Ghani et al., 2006). The state where abnormalities in glucose metabolism are present but blood glucose concentration is below the threshold for type 2 diabetes diagnosis is termed pre-diabetes (Vendrame and Gottlieb, 2004). The exact definition of pre-diabetes and type 2 diabetes are frequently debated and have been subject to constant revision over time (Genuth et al., 2003, Davidson et al., 2003, Schriger and Lorber, 2004). Pre-diabetes is defined as one of the following states: when the individual has high fasting plasma glucose concentration with a normal response to a glucose load; an abnormal postprandial glucose excursion but normal fasting glucose concentration; or a combination of high fasting glucose and abnormal response to a glucose load (Abdul-Ghani et al., 2006). Accordingly, the American Diabetes Association defines pre-diabetes based on any one of the following three criteria: an HbA1c of 5.7-6.4%, a fasting blood glucose concentration of 5.6-6.9 mmol/L and/or an oral glucose tolerance test of 7.8-11.0 mmol/L at 2 hours (American Diabetes Association, 2011). In addition, many patients with

prediabetes also have the metabolic syndrome, which is characterised by hypertension, abdominal obesity and dyslipidaemia (Cornier et al., 2008).

As insulin resistance develops, normally insulin sensitive tissues do not respond adequately to insulin (Shulman, 2000) and blood glucose concentration is not as tightly regulated. This results in large excursions in blood glucose following glucose ingestion due to a reduction in insulin-mediated glucose uptake in peripheral tissues and failure of insulin-mediated suppression of hepatic glucose production (Abdul-Ghani et al., 2006). In the initial period of pre-diabetes, the pancreatic β cells compensate for the peripheral insulin resistance by secreting more insulin, resulting in hyperinsulinaemia. Over time, the increased demand exerted on the β cells by the continuous hyperinsulinaemia leads to more severe insulin resistance and ultimately β cell failure and apoptosis. This causes plasma glucose concentrations to be chronically elevated, eventually leading to type 2 diabetes. The American Diabetes Association (2011) diagnostic definition for type 2 diabetes is a fasting plasma concentration of >7.0 mmol/L or a plasma concentration of >11.1 mmol/L post 2 hour 75g oral glucose tolerance test. Chronic hyperglycaemia is associated with macrovascular and microvascular complications including nephropathy, neuropathy, and retinopathy that over time can lead to severe outcomes including coronary heart disease, kidney failure, limb amputations and blindness. The following sections will now outline the mechanisms leading to the development of insulin resistance.

2.3.1. Mechanisms of Insulin Resistance

Skeletal muscle insulin resistance cannot be explained by a single pathophysiological mechanism as it involves dysregulation to numerous cellular and metabolic pathways which will be outlined below and summarised in Figure 2.2. Key features of insulin resistance include; 1) decreased delivery of insulin and glucose to

the myocyte due to impaired insulin-mediated dilation of the muscle microvasculature and decreased capillary density, 2) impaired lipid metabolism and accumulation of fatty acid metabolites, and 3) impaired insulin signalling at the myocyte leading to impaired GLUT4 translocation.

2.3.1.1. Microvascular Insulin Resistance and Reduced Capillary Density

Insulin exerts a physiological action at all levels of the arterial vascular tree and as such, insulin resistance is accompanied by dysfunction at each level (Barrett and Liu, 2013). In the conduit arteries it accelerates atherosclerosis; in resistance vessels it enhances risk for hypertension and increases vascular stiffness; in small arterioles it can impair NO-mediated vasodilation; and capillary rarefaction diminishes the available surface for nutrient exchange, thereby limiting insulin access to the tissue and promoting metabolic insulin resistance (Chantler and Frisbee, 2015, Labazi and Trask, 2017, Muniyappa et al., 2007). Microvascular dysfunction may have effects on insulin-mediated changes in muscle perfusion and glucose metabolism which would lead to increased postprandial hyperglycaemia, hyperinsulinaemia and hypertriglycaemia, which eventually lead to type 2 diabetes (Wagenmakers et al., 2016).

The importance of insulin's microvascular actions have been demonstrated in studies using animal models of chronic insulin resistance showing a reduced microvascular response during insulin infusion and impaired insulin-mediated glucose uptake (Clerk et al., 2007, Wallis et al., 2002, St-Pierre et al., 2010). de Jongh et al. (2004) examined skin microvascular function in 16 lean and 12 obese healthy women in the basal and hyperinsulinaemic state. Impaired microvascular function was associated with decreased insulin sensitivity and increased blood pressure. Therefore, it is anticipated that the blunted capillary perfusion seen in

obese individuals would contribute at least in part to the reduced insulin-mediated glucose disposal.

Endothelial function is dependent on NO bioavailability (McAllister and Laughlin, 2006) which in turn is determined by the balance between NO synthesis and scavenging by superoxide anions and related reactive oxygen species (ROS). With insulin resistance, there is an altered balance between the formation and quenching of NO by superoxide anions and other ROS (Silver et al., 2007). High free fatty acid (FFA) levels associated with insulin resistance increase ROS production in the vasculature, with the two primary sources being NAD(P)Hoxidase (NAD(P)Hox) and the mitochondrial electron transport chain. Obesity also causes vascular inflammation leading to gene induction of NAD(P)Hox and activation of NOX2 by $p47^{phox}$ (Silver et al., 2007). This then leads to production of superoxide anions which will scavenge NO therefore reducing NO bioavailablity for vasodilation of the skeletal muscle microvasculature. This occurs by superoxide anions interacting with NO to generate peroxynitrite, which subsequently reduces NO bioavailability. Peroxynitrite can then oxidise tetrahydrobiopterin to inhibit PGI_2 and alter NO-induced cGMP signalling in VSMCs (Munzel et al., 2005).

With obesity, exposure of the vasculature to high levels of FFA initiates cellular processes including impaired insulin signalling (Belfort et al., 2005) and oxidative stress (Inoguchi et al., 2000) which lead to vascular insulin resistance. High FFA levels lead to accumulation of diacylglycerol (DAG), ceramides and long-chain fatty acyl-CoAs (LCFACoA) in the endothelial cells of the skeletal muscle microvasculature (Rask-Madsen and King, 2005, Symons and Abel, 2013). These lipid metabolites activate serine kinases such as protein kinase C (PKC) and inhibitory kB kinase (IKKβ), that regulates nuclear factor-κB (NF-κB) which is a transcription factor associated with inflammation (Itani et al., 2002). Activation of PKCβ1 and β2 result in increased serine phosphorylation of IRS-1 leading to reduced insulin-stimulated Akt and eNOS (Naruse et al., 2006).

Insulin resistance, obesity and endothelial dysfunction are also characterised by increased inflammation (Fernandez-Real and Ricart, 2003). Visceral adipose tissue secretes a wide range of hormones including leptin, adiponectin, TNF-α, IL-6, resistin, angiotensinogen and plasminogen activator inhibitor-1 that affect vascular metabolism. A full overview of the effects of inflammation on vascular insulin resistance is beyond the scope of this introduction so interested readers are referred to a review by Muniyappa et al. (2007). Briefly, one key mechanism is that elevated plasma triglyceride (TG; chylomicron-TG and VLDL-TG) concentrations in the insulin resistant state leads to increased ROS production, which activates NF-κB to stimulate release of proinflammatory cytokines such as TNF-α and IL-6. TNF-α activates serine kinases including JNK, IKKβ and IL-Iβ receptor associated kinase (Nguyen et al., 2005). These directly or indirectly increase serine phosphorylation of IRS1/2 leading to decreased PI3K activity in response to insulin, ultimately leading to decreased NO bioavailability (Kim et al., 2001).

Under insulin resistant conditions, the PI3K pathway within the endothelium is selectively impaired, diminishing NO production (Cusi et al., 2000). However, the MAPK pathway remains intact so that ET-1 production is maintained and the balance between NO and ET-1 production becomes skewed (Potenza et al., 2005). The increased ET-1 production due to enhanced MAPK-dependent functions (Piatti et al., 1996) further promotes vasoconstriction and opposes residual NO activity (Younk et al., 2014). Therefore, the compensatory hyperinsulinaemic state associated with insulin resistance has important pathophysiological implications due to the increased ET-1 induced vasoconstriction.

Obesity and inactivity are also associated with reduced skeletal muscle capillary density which limits skeletal muscle perfusion (Hoier and Hellsten, 2014). Microvascular density is important in determining insulin sensitivity and $\dot{V}O_{2max}$ as it determines maximal capillary surface area available for exchange of insulin, oxygen

and nutrients (Frisbee, 2007). Capillary density is much higher in active than sedentary individuals (Blomqvist and Saltin, 1983) and there is a strong correlation between muscle capillary density with $\dot{V}O_{2max}$ and insulin sensitivity (Lillioja et al., 1987). In **Chapter 5** of this thesis the adaptations to the skeletal muscle microvasculature during 12 weeks of Home-HIT will be investigated. This will provide information on the key mechanisms in the microvasculature underpinning the increased insulin sensitivity and aerobic capacity in obese individuals with elevated CVD risk.

2.3.1.2. Lipid Induced Insulin Resistance and the Lipid Overflow Hypothesis

The second major mechanism for obesity-related insulin resistance that will be discussed in this introduction is lipid induced insulin resistance in the skeletal muscle. Following a meal in normal healthy individuals, the rise in plasma glucose concentration is controlled through coordinated mechanisms that increase plasma glucose disposal and suppress endogenous glucose production. This glucose 'buffering' minimises the exposure of tissues to the potentially damaging effects of hyperglycaemia that can lead to long-term vascular complications. In the same way, it has been argued that adipose tissue buffers the flux of fatty acids into the circulation during the postprandial period (Frayn, 2002). If this buffering process is impaired, as happens with obesity, tissues such as the liver, skeletal muscle and pancreatic β cells are exposed to excessive concentrations of TGs which can accumulate in the form of triacylglycerol (TAG), leading to insulin resistance via a complex array of metabolic abnormalities (Lewis et al., 2002, Frayn, 2002). The series of events by which failure of adipose tissue to buffer the influx of fatty acids leads to insulin resistance has been described by the "lipid overflow hypothesis" (Frayn, 2002).

In the fasted state, insulin concentrations are low and plasma TG output from adipose tissue is high. Following a mixed meal, however, there is a rise in plasma

glucose concentrations resulting in insulin secretion from pancreatic β cells which has three main effects to buffer the FFA flux: 1) reduce the activity of hormone sensitive lipase (HSL) activity in the adipose tissue to suppress lipolysis and the release of FFAs into the circulation; 2) activation of lipoprotein lipase (LPL) in the capillary bed of adipose tissue, which stimulates FFA uptake into the adipose tissue for storage as TAG; 3) decreased LPL activity in the capillaries of skeletal muscle which reduces FFA uptake into the skeletal muscle. These mechanisms enable adipose tissue to buffer the lipid flux following a meal and protect non-adipose tissues such as the liver, skeletal muscle, heart and pancreatic β cells from the potentially damaging effects of elevated FFAs (Frayn, 2002).

In obesity, the ability of adipose tissue to buffer the lipid flux is compromised (Frayn, 2002). The relative insulin resistance means that insulin does not increase LPL activity in adipose tissue following ingestion of a meal which reduces the capacity of adipose tissue to buffer the lipid flux causing FFAs to 'spillover' into non-adipose tissues. In addition, there is a reduced capacity of insulin to suppress LPL activity in skeletal muscle leading to increased uptake of FFAs into this tissue. The accumulation of lipid in skeletal muscle (intramuscular triglyceride; IMTG) observed in obesity and type 2 diabetes is associated with insulin resistance (Goodpaster et al., 2001, Pan et al., 1997).

At this point it is important to note that endurance-trained athletes have equal or even higher IMTG content than obese individuals or type 2 diabetes patients, despite being insulin sensitive (Goodpaster et al., 2001). This phenomenon has been termed the "athletes' paradox" (Goodpaster et al., 2001). The elevated IMTG storage in endurance-trained individuals appears to represent an important metabolic adaptation to endurance training (van Loon, 2004, Amati et al., 2011). Indeed, the IMTG pool makes a significant contribution to total substrate oxidation during a bout of endurance exercise (van Loon et al., 2003). Research suggests that

skeletal muscle lipid oxidation is influenced by the spatial and functional interactions between mitochondria and lipid droplets. This appears to be an adaptation that occurs with training, as studies using electron microscopy have shown that the number of lipid droplets in contact with the mitochondria increases following SIT (Shepherd et al., 2017) and endurance training (Tarnopolsky et al., 2007). Therefore, in trained individuals with a high mitochondrial content, the capacity to oxidise plasma FFA and IMTG as a fuel source during exercise is high. Fundamentally, the regular breakdown and subsequent re-synthesis of IMTG in the post-exercise period in endurance-trained individuals means that the turnover rate of the IMTG pool is high, and this underpins the high insulin sensitivity in this population.

Several experimental approaches have been taken to determine the mechanisms underpinning lipid-induced insulin resistance. Raising plasma FFA concentrations through infusion of intralipid and heparin or prolonged fasting (which is accompanied by an increased release of fatty acids from endogenous lipid stores) induces insulin resistance. Furthermore, cross-sectional studies comparing obese individuals and/or type 2 diabetes patients to healthy insulin-sensitive controls also offer insight into the mechanisms of lipid-induced insulin resistance. As a result of these studies, the current belief is that a functional imbalance exists between IMTG storage, lipolysis and fatty acid oxidation, leading to the accumulation of intramuscular lipid metabolites including LCFA-CoA, DAG and ceramides which are responsible for the impairment in insulin action (Amati et al., 2011, Itani et al., 2002). This imbalance is largely driven by a poor capacity of sedentary obese and/or type 2 diabetic individuals to break down and oxidise IMTG during exercise (Moro et al., 2008, Shaw et al., 2010). Indeed, studies using stable-isotope tracers have shown that IMTG contribute 40-50% of total fat oxidation during exercise in trained individuals, but IMTG makes a negligible contribution to fat oxidation in obese individuals and type 2 diabetes patients (van Loon et al., 2003, Blaak et al., 2000,

Schrauwen et al., 2002). In addition, a sedentary lifestyle leads to reduced mitochondria content and function which contributes to insulin resistance (Wagenmakers, 2005). Mitochondrial dysfunction is proposed to decrease the rate of β-oxidation, explaining the accumulation of intramuscular lipid metabolites that disrupt insulin signalling (Lowell and Shulman, 2005). However, it was also proposed by Koves et al. (2008) that lipid-induced insulin resistance may be due to excessive rather than reduced β-oxidation. This model suggested that oversupply of lipid to the mitochondria with obesity drives an increase in mitochondrial β-oxidation that exceeds the capacity of the Krebs cycle and electron transport chain leading to incomplete fatty acid oxidation and intra-mitochondrial accumulation of metabolites, mitochondrial stress and cellular dysfunction.

The accumulation of fatty acid metabolites (including LCFA-CoA, DAGs and ceramides) is combined with increased plasma levels of inflammatory cytokines and local inflammation to inhibit the proximal insulin signalling cascade leading to reduced GLUT4 translocation to the plasma membrane (Hulver and Dohm, 2004). DAG is an intermediate of IMTG synthesis and hydrolysis and increases with insulin resistance in humans (Itani et al., 2002). However, there is also data showing that athletes have greater myocellular DAG than obese individuals (Amati et al., 2011), suggesting that the cellular localisation of DAG is more important in the development of insulin resistance with obesity. Indeed, Bergman et al. (2012b) found that only saturated DAG in skeletal muscle membranes was related to insulin resistance. DAG activates novel protein kinase C (PKC) isoforms (Itani et al., 2002, Bergman et al., 2012a) which inhibit the action of IRS-1 via serine phosphorylation leading to downstream inactivation of the insulin signalling cascade (Shaw et al., 2010, Morino et al., 2006).

With obesity and type 2 diabetes, ceramide concentration is elevated, and in particular, long-chain C18:0 and C16:0 ceramide species have been identified as

potentially critical in human insulin resistance (Tonks et al., 2016, Perreault et al., 2018). Ceramide accumulation in the mitochondria is inversely related to insulin sensitivity as it may impede mitochondrial respiration (Perreault et al., 2018). Ceramide accumulation inhibits the insulin signalling pathway downstream of PI3K through the activation of protein phosphatase 2A which phosphorylates and inactivates Akt/PKB (Amati et al., 2011), meaning TBC1D4 and subsequently GLUT4 activation are inhibited (Mukhopadhyay et al., 2009).

A full description of lipid induced insulin resistance is beyond the scope of this introduction; therefore, interested readers are referred to reviews by Shaw et al. (2010) and Tumova et al. (2016) for more information. Together it appears that the accumulation of IMTG and lipid metabolites per se do not cause insulin resistance. Rather, it is the reduced turnover of the IMTG pool due to inactivity and reduced access of the lipid droplets to the mitochondria for oxidation suggesting impaired metabolic flexibility. With exercise training there will be increased IMTG turnover resulting in a reduction in the accumulation of lipid metabolites such as ceramides, LCFA-CoA and DAG that can impair insulin signalling at the IRS-1 and Akt/PKB (Shulman, 2014, Amati et al., 2011, Shaw et al., 2010), eventually leading to improved insulin sensitivity (Shepherd et al., 2017). Exercise training should therefore be utilised as a strategy to improve insulin sensitivity.

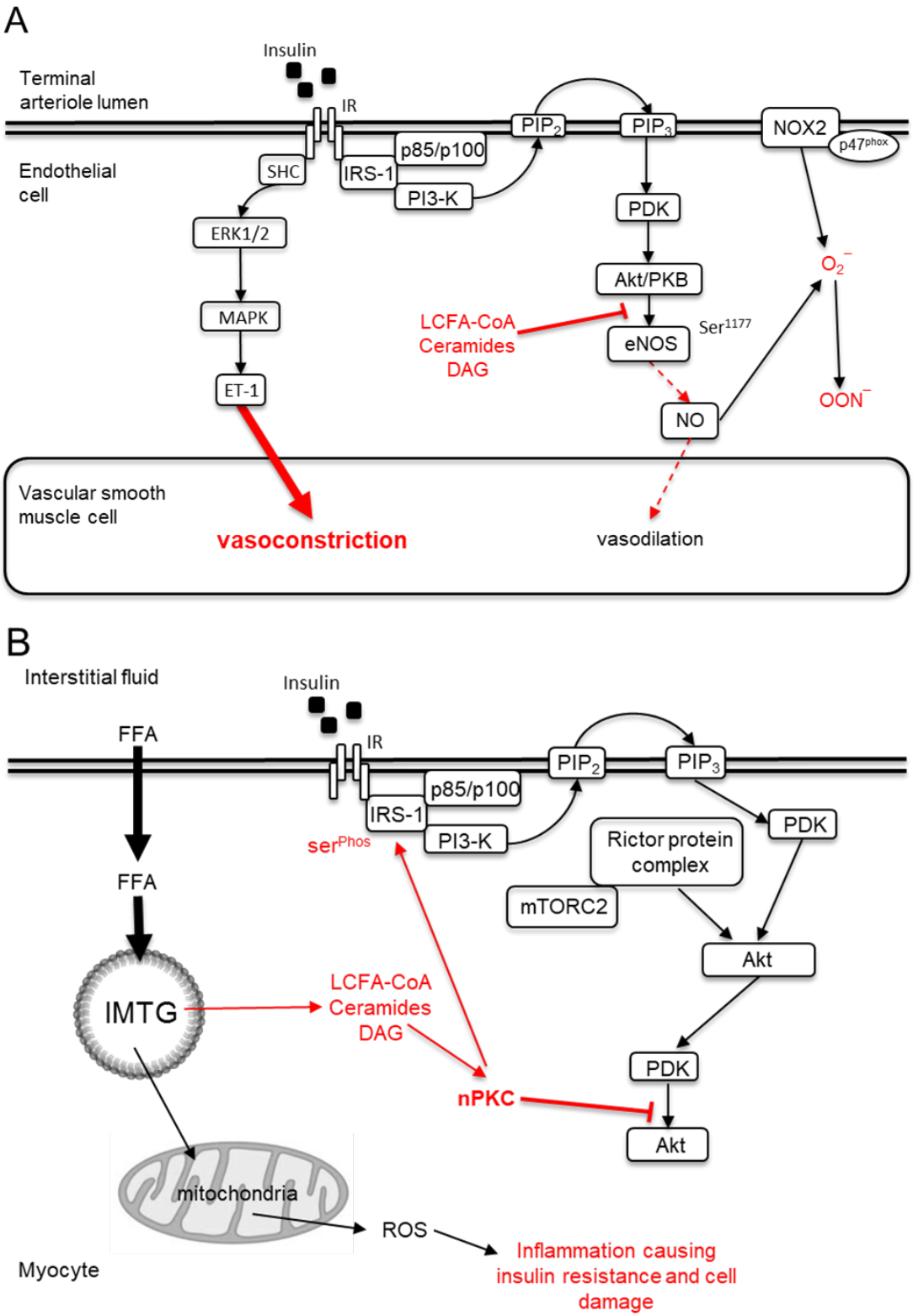

Figure 2.2. Mechanisms of insulin resistance

A) Shows the impaired insulin signalling in the vascular endothelial cell leading to impaired nitric oxide (NO)-mediated vasodilation and increased endothelin-1 (ET-1) induced vasoconstriction. Obesity increases NOX2 activation which leads to production of superoxide anions (O_2^-) which in turn interact with NO to generate peroxynitrite (OON^-) which reduces NO bioavailability. B) Shows impairments in

insulin signalling at the myocyte resulting in impaired glucose uptake. Under insulin resistant conditions, the PI3K pathway within the vascular endothelium is selectively impaired, diminishing NO production. However, the MAPK pathway remains intact so that ET-1 production is maintained which further promotes vasoconstriction and opposes residual NO activity. In the skeletal muscle, an increased FFA flux leads to intramuscular triglyceride (IMTG) accumulation in this tissue. An imbalance in the storage, breakdown and oxidation of IMTG leads to accumulation of lipid metabolites which impair the insulin signalling cascade resulting in reduced GLUT4 translocation. Excess lipid drives an increase in mitochondrial β oxidation which without an increase in energy demand leads to reactive oxygen species (ROS), inflammation and cell damage.

2.4. Exercise Training as an Agent to Combat Metabolic Disease Risk

A sedentary lifestyle is an important modifiable risk factor for the development of type 2 diabetes and CVD. Regular physical activity slows the progression of type 2 diabetes and cardiovascular events due to the beneficial effects on body weight, insulin sensitivity, glycaemic control, lipid profile, endothelial function and aerobic capacity (see Section 2.4.2.). Large cohort studies consistently show that low aerobic fitness and physical activity levels predict an increased risk of cardiovascular mortality and type 2 diabetes (Kohl et al., 1992, Helmrich et al., 1991, Manson et al., 1991, Manson et al., 1992) and interested readers are directed to a review by Bassuk and Manson (2005) on epidemiological observations. For example, the Nurses' Health Study showed that among 70,000 initially healthy women aged 40-65 years that walking briskly for at least 2.5 hours per week (i.e. 30 minutes per day for 5 days a week) was associated with a 25% reduction in type 2 diabetes over an 8-year follow-up period compared to those reporting no vigorous exercise (Hu et al., 1999).

Clinical trials using large cohorts have demonstrated that lifestyle interventions involving diet and exercise reduce the incidence of type 2 diabetes in people with impaired glucose tolerance (Tuomilehto et al., 2001, Lindstrom et al., 2003, Eriksson and Lindgarde, 1991, Eriksson and Lindgarde, 1998). In the Finnish Diabetes Prevention Study (Lindstrom et al., 2003), 522 overweight individuals with

impaired glucose tolerance were randomly assigned to a lifestyle intervention or control condition. The intervention group aimed to perform moderate-intensity exercise at least 30 minutes per day alongside a diet intervention. Follow-up showed that incidence of type 2 diabetes in the intervention group was less than half that of the control group. Similarly, in the Malmo study (Eriksson and Lindgarde, 1991, Eriksson and Lindgarde, 1998), 161 people with impaired glucose tolerance participated in a diet and exercise intervention and were compared after 6 years with 56 individuals that declined to take part in the same intervention. The incidence of type 2 diabetes after 6 years was 11% in the intervention group and 21% in the controls (Eriksson and Lindgarde, 1991). After 12 years of follow-up, mortality rate in the intervention group was less than half that of the control group (Eriksson and Lindgarde, 1998).

Altogether, these large-scale studies provide strong evidence that regular, long term exercise reduces the risk of cardio-metabolic disease and mortality. Broadly speaking there are two aspects by which exercise enhances glucose homeostasis. First, the effects of an acute bout of exercise to enhance glucose uptake and improve post-exercise insulin sensitivity, and second, the longer term molecular adaptation with chronic exercise training leading to greater insulin sensitivity.

2.4.1. Effect of an Acute Exercise Bout on Insulin Sensitivity

Exercise (muscle contractions) has two main effects on muscle glucose metabolism. First, acute exercise stimulates increased glucose uptake via GLUT4 translocation, and second, a single bout of exercise increases insulin sensitivity for up to 48 hours to facilitate rapid glycogen resynthesis (Wojtaszewski et al., 1997, Richter et al., 1984). From an evolutionary perspective, increased insulin sensitivity resulting in glycogen synthesis may be necessary to restore energy homeostasis (Jensen and

O'Rahilly, 2017), which would ultimately be beneficial during 'fight-or-flight' situations. However, in today's obesogenic environment, exercise is an effective way to improve glycaemic control in insulin resistant individuals as blood glucose levels will be reduced after a bout of exercise (Wojtaszewski and Richter, 2006). This prolonged increase in insulin sensitivity following each exercise bout is a driver for the glucoregulatory benefits of regular endurance exercise (Morrison et al., 2017, Segal et al., 1991). Segal et al. (1991) investigated the effects of 12 weeks of exercise training (cycling at 70% $\dot{V}O_{2max}$ 4 hours per week) on insulin action and glucose metabolism where bodyweight and body fat content were held constant in lean, obese and type 2 diabetic men. They found that despite the 27% increase in $\dot{V}O_{2max}$, the training had no effect on insulin-stimulated glucose disposal measured 4-5 days after the last training session. More recently, Morrison et al. (2017) showed that postprandial glucose and insulin responses were reduced to the same extent following acute and chronic training. These observations suggest that the glucoregulatory benefits of endurance exercise are largely attributed to the residual effects of the last exercise bout.

Establishing the mechanisms by which exercise improves muscle insulin sensitivity has proved difficult since Richter et al. (1984) first observed that exercise had an effect on muscle insulin sensitivity 36 years ago, and this is still not completely understood. Wojtaszewski et al. (2000) excluded the proximal portion of the insulin signalling cascade from contributing to the increase in muscle insulin sensitivity 4-48 hours after a single bout of one-legged knee extensor exercise. They found that insulin-stimulated glycogen synthase activity and glucose uptake in exercised muscle was not associated with upregulation of insulin signalling through the insulin receptor, Akt and GSK-3. A recent paper by Kjobsted et al. (2017) provided evidence the AMPK-TBC1D4 signalling axis is the likely mediating factor for improved insulin sensitivity up to 48 hours following muscle contractions.

Kjobsted et al. (2017) showed that AMPK activity is required for muscle contraction to increase insulin sensitivity as deletion of the two catalytic subunits (α_1 and α_2) prevented an exercise induced increase in insulin sensitivity. Kjobsted et al. (2017) also found that improved insulin sensitivity following contractions was not associated with enhanced glycogen synthase activity or proximal insulin signalling, which provides support for the study by Wojtaszewski et al. (2000).

2.4.2. Adaptations Underpinning Exercise Training Induced Increases in $\dot{V}O_{2max}$ and Insulin Sensitivity

A high aerobic capacity ($\dot{V}O_{2max}$) and insulin sensitivity are key features of an optimal healthy phenotype so will be a major focus of this thesis. Aerobic exercise capacity is the strongest predictor of mortality risk even compared to more clinical variables or established risk factors such as hypertension, smoking or diabetes (Myers, 2003). Increasing $\dot{V}O_{2max}$ through exercise training can reduce the risk of mortality by an equivalent (Paffenbarger et al., 1993) or greater magnitude (Blair et al., 1995) compared to modifying other risk factors such as giving up smoking. Insulin sensitivity is also important for reducing the risk of clinical events and the two factors are positively correlated (Goodpaster et al., 2001). This section will cover the effects of exercise training on mitochondrial density, capillary density, lipid metabolism and insulin signalling as these are key factors within the skeletal muscle that are associated with improvements in $\dot{V}O_{2max}$ and insulin sensitivity. Other training induced adaptations in cardiac muscle, blood composition and arterial structure are also important. However, these cardiovascular adaptations are beyond the scope of this introduction so interested readers are referred to Hellsten and Nyberg (2015).

Increased mitochondrial protein content is a major training adaptation that increases skeletal muscle oxidative potential (Holloszy, 1967, MacInnis and Gibala,

2017). Skeletal muscle mitochondria regulates substrate metabolism during submaximal exercise, with greater mitochondrial content promoting an increased reliance on fat oxidation and a proportional decrease in carbohydrate oxidation (Egan and Zierath, 2013). This results in reduced glycogen degradation and lactate production at a given exercise intensity, while increasing the lactate threshold and allowing individuals to exercise for longer and at greater intensities. Mitochondrial biogenesis reflects an increase in the abundance of proteins involved in mitochondrial ATP production, the Krebs cycle, mobilisation, transport and oxidation of fatty acids, glycolytic metabolism, antioxidant capacity, glucose transport and glycogen synthesis (Egan and Zierath, 2013). The increase in mitochondrial content appears to be a relatively short-term adaptation to training and the reader is directed to a paper by Egan and Zierath (2013) for a detailed review on the signalling pathways involved in mitochondrial biogenesis. Very briefly, a single bout of MICT activates signalling pathways to induce transient mRNA expression, and the repeated activation of these pathways leads to increases in mitochondrial protein content (Coffey and Hawley, 2007). Egan et al. (2013) investigated the time-course of adaptation of mitochondrial mRNA expression and protein content during aerobic training. They found that there was increased mRNA expression of genes including PGC1-α and EERα after one session which was proceeded by progressive increases in cytochrome oxidase IV (COX IV) expression after 2 weeks of training. Increased mitochondrial protein content is also important in regulation of fatty acid turnover with training which will reduce the accumulation of lipid metabolites that are implicated in impairment in the insulin signalling cascade (Shulman, 2000). Therefore, mitochondrial biogenesis with aerobic exercise training is associated with improved insulin sensitivity.

Microvascular density has an important role in determining aerobic capacity and insulin sensitivity as it determines the maximal surface area available for exchange of insulin, nutrients and oxygen (Akerstrom et al., 2014, Hellsten and

Nyberg, 2015). Capillary density is higher in active individuals and there is a strong positive correlation between skeletal muscle capillary density and $\dot{V}O_{2max}$ and insulin sensitivity. Exercise training increases skeletal muscle capillarisation in human skeletal muscle (Andersen and Henriksson, 1977, Wagenmakers, 2016) and the mechanisms are reviewed in detail by Hoier and Hellsten (2014).

Endurance training increases microvascular eNOS content in lean sedentary and obese individuals (Cocks et al., 2013, Cocks et al., 2016), insulin induced ser^{1177} phosphorylation and vasodilatory response capacity of the skeletal muscle microvasculature to insulin. Oxidative stress is a systemic feature of obesity and excessive ROS production can result in apoptosis and increased cellular permeability which may promote inflammation, endothelial dysfunction and vascular remodelling. NAD(P)Hox is considered a prominent source of vascular derived ROS that promotes vascular dysfunction (Weseler and Bast, 2010). NOX2 which is a catalytic subunit of NAD(P)Hox and its activation by $p47^{phox}$, has been suggested to be a major source of superoxide anion production in obese individuals (Silver et al., 2007, La Favor et al., 2016). These superoxide anions will scavenge NO thereby reducing the bioavailability of NO for vasodilation of the skeletal muscle microvasculature. Four weeks of MICT increased capillarisation and eNOS/NAD(P)Hox balance in previously sedentary obese (Cocks et al., 2016) and lean (Cocks et al., 2013) males which was associated with improved insulin sensitivity and $\dot{V}O_{2max}$.

Cross-sectional studies have shown that IMTG content is greater in trained compared to obese or type 2 diabetic individuals (Goodpaster et al., 2001) and endurance trained athletes have a higher IMTG fractional synthetic rate compared to sedentary individuals (Bergman et al., 2010). Exercise training increases the capacity to oxidise fat during exercise, which decreases reliance on carbohydrate (van Loon, 2004). Longitudinal training studies consistently show that there is an

increase in IMTG utilisation during moderate-intensity exercise with training (Schrauwen et al., 2002, De Bock et al., 2008, Van Proeyen et al., 2011, Phillips et al., 1996a) due to greater expression of enzymes responsible for IMTG hydrolysis. A single bout of endurance exercise has been shown to prevent fatty acid induced insulin resistance during lipid infusion on the following day (Schenk and Horowitz, 2007). This was accompanied by increased expression of enzymes involved in triglyceride synthesis and reduced accumulation of fatty acid metabolites. Increased mitochondrial density combined with an increased IMTG content following training suggests an adaptation whereby IMTG-containing lipid droplets are in closer proximity to the mitochondrial reticulum, which underpins the training-induced increase in IMTG utilisation. Indeed, four weeks of MICT increased the number of lipid droplets in contact with the mitochondria in obese males (Shepherd et al., 2017). This adaptation likely enhanced the capacity for IMTG utilisation during exercise, and will result in reduced accumulation of lipid metabolites such as ceramides, LCFA-CoA and DAGs that can impair insulin signalling (Shulman, 2014, Amati et al., 2011, Shaw et al., 2010), thereby contributing to improved insulin sensitivity. In support, Shepherd et al. (2017) also reported a reduction in muscle ceramide concentrations alongside an improvement in insulin sensitivity following 4 weeks of MICT in obese individuals.

Regular exercise training leads to increased expression and/or activity of key signalling proteins involved in the regulation of glucose uptake and metabolism (Hawley and Lessard, 2008). Part of the improved glycaemic control achieved through exercise training may be due to the cumulative effects of acute exercise bouts which firstly reduce plasma glucose concentration through contraction-mediated glucose uptake and secondly improve insulin sensitivity of working muscle up to 48 hours after the exercise bout. With regular exercise training there are

adaptations that result in reduced basal and glucose-stimulated insulin levels, improved intracellular insulin signalling and increased GLUT4 protein content.

Defects in insulin signal transduction through the IRS-1/PI3K pathway are associated with reduced GLUT4 translocation in insulin resistant skeletal muscle (Zierath et al., 1996, Bjornholm et al., 1997). Therefore, the metabolic adaptations with exercise training may involve changes in expression of proteins involved in glucose transport. Chronic exercise training has been shown to result in higher rates of tyrosine phosphorylation of molecules of the insulin signalling cascade in healthy and insulin-resistant humans (Frosig et al., 2007, Consitt et al., 2013). Exercise training has been shown to stimulate PI3K in skeletal muscle (Kirwan et al., 2000, Chibalin et al., 2000) and trained individuals have increased insulin-stimulated PI3K activation than untrained suggesting a positive association between PI3K activation and $\dot{V}O_{2max}$ (Kirwan et al., 2000). As PI3K is an important step in insulin-stimulated GLUT4 translocation, this is likely to be one step that regular physical activity improves insulin signalling. Improvements in whole-body insulin-mediated glucose uptake after exercise have been attributed to an increase in intracellular signalling via PI3K activity (Houmard et al., 1999, Kirwan et al., 2000). This is clinically relevant because PI3K activity is decreased in skeletal muscle from insulin resistant participants and in those with type 2 diabetes (Goodyear et al. 1995). Frosig et al. (2007) investigated the effect of 3 weeks of leg extensor endurance exercise in 8 healthy men. Training increased activity and phosphorylation of Akt1 and AS160 alongside an increase in insulin sensitivity. However, in contrast to expectations, they did not find increased IRS-1 associated PI3K activity with training.

Increased skeletal muscle GLUT4 content is associated with improved insulin action, as shown in animal models overexpressing GLUT 4 (Ren et al., 1995, Hansen et al., 1995). Increased GLUT4 levels is an adaptation to exercise training in humans leading to improved insulin sensitivity (Phillips et al., 1996b, Kraniou et al.,

2004) and there is a rapid decline in GLUT4 with inactivity (McCoy et al., 1994). In humans, GLUT4 mRNA concentration increases in skeletal muscle within 3 hours after a single exercise bout (Neufer and Dohm, 1993) which is followed by increases in GLUT4 protein content after 16-24 hours (Ren et al., 1994). This will not lead to acute changes in insulin sensitivity, but with repeated exercise bouts over time (exercise training) adaptations to physical activity to include more long-lasting increases in insulin sensitivity (Ojuka et al., 2012). Bradley et al. (2014) showed that GLUT4 protein content increased following 6 weeks of SIT and endurance training in previously sedentary, lean males and this was associated with similar improvements in insulin sensitivity in both groups.

The aforementioned markers of metabolic health are all important for a metabolically healthy, insulin-sensitive phenotype and are improved with regular exercise training. For this reason, **Chapter 4** and **5** used a wide range of physiological tests to comprehensively establish the effectiveness of Home-HIT in obese individuals with elevated CVD.

2.5. Type 1 Diabetes

Unlike obesity-induced insulin resistance and type 2 diabetes which is largely driven by lifestyle factors, type 1 diabetes, previously known as insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes, is a chronic inflammatory autoimmune disease whereby the insulin-producing β-cells of the islets of Langerhans of the pancreas are destroyed (Atkinson and Eisenbarth, 2001). Type 1 diabetes accounts for 10-15% of all cases of diabetes (>16 million cases worldwide), affecting ~400,000 people in the UK (jdrf.org.uk/about-type-1-diabetes) at a cost of ~£1.8 billion to the NHS per annum (Hex et al., 2012). The incidence of type 1 diabetes is increasing annually by 3-5%, possibly due to environmental factors such as the obesity epidemic or increases in human hygiene (Peng and Hagopian, 2006, Chapman et al., 2012,

Bach and Chatenoud, 2012), although mechanisms for this are speculative at present.

The classical symptoms of type 1 diabetes include weight loss due to uncontrolled glucose, protein and lipid metabolism, as well as glucose excretion in the urine; increased urination (polyuria) due to glucose in the urine; and increased thirst (polydipsia) due to increased urination and changes in the osmolality of extracellular fluid (Daneman, 2006). Type 1 diabetes increases the risk of numerous acute and long-term complications because of the lifetime exposure to erratic blood glucose levels (Chimen et al., 2012). Acute complications include diabetic ketoacidosis and hypoglycaemia, which can be potentially life threatening. Long-term complications include neuropathy, nephropathy, retinopathy, heart disease, stroke and foot ulcers, which in severe cases may lead to amputation. Type 1 diabetes shortens life expectancy by 15-20 years and risk of death is 3-4 times higher than for non-diabetics (Team, 2007, Dawson et al., 2008). This is particularly true in young females, mostly because their protection from CVD is lost and they have higher levels of centrally distributed obesity compared to non-diabetic females (Narendran et al., 2015, Krishnan et al., 2012).

The following sections will discuss the importance of regular exercise for people with type 1 diabetes, as physical inactivity and obesity can also increase the risk of insulin resistance and vascular complications in this population.

2.5.1. The Importance of Exercise for People with Type 1 Diabetes

Regular exercise is recommended for those with type 1 diabetes for maintenance of overall health and prevention of macrovascular and microvascular complications, which are a major cause of mortality and morbidity (Chimen et al., 2012, Moy et al., 1993, Wasserman and Zinman, 1994, Devaraj et al., 2007). However, people with type 1 diabetes tend to be at least as inactive as the general population, with a large

percentage of patients not maintaining a healthy body mass or meeting physical activity guidelines (Tielemans et al., 2013, Makura et al., 2013). The majority of patients living with type 1 diabetes do not have a healthy bodyweight as ~60% are overweight or obese (Bohn et al., 2015). Large scale studies such as the Swedish National Diabetes Registry found that among the 21,000 type 1 diabetic adults on their record, that obesity was significantly associated with increased risk for heart failure (Vestberg et al., 2013), and Price et al. (2014) found that obesity was associated with retinopathy and macrovascular disease in those with type 1 diabetes. Inactive people with type 1 diabetes are also at risk of the metabolic syndrome, sometimes referred to as “double diabetes” (Cleland, 2012), as insulin resistance is common and inversely related to HbA1c (Yki-Jarvinen and Koivisto, 1986). Data from the Finnish Diabetic Nephropathy study showed that the prevalence of the metabolic syndrome was 38% in men and 40% in women with type 1 diabetes (Thorn et al., 2005). Obesity and incidence of the metabolic syndrome is rising in the type 1 diabetic population (Chillaron et al., 2014), resulting in an increase in the incidence of cardiovascular events and diabetic complications.

Insulin resistance is common in people with type 1 diabetes and the incidence is rising, mirroring the trend in the general population and reflecting the rising rates of obesity (Kilpatrick et al., 2007, McGill et al., 2008). This is important given that insulin resistance is an additional independent risk factor for micro- and macro-vascular complications in those with type 1 diabetes (Orchard et al., 2003). There is likely overlap in terms of the mechanisms for insulin resistance in type 1 and 2 diabetes, including increased IMTG content (Perseghin et al., 2003) and mitochondrial dysfunction (Kacerovsky et al., 2011). However, hyperglycaemia alone is unable to explain the high prevalence of insulin resistance observed in those with type 1 diabetes (Fasching et al., 1993, Kacerovsky et al., 2011, Bergman et al., 2012a). It has been speculated that chronic exogenous insulin use is an important factor, as exposure to a long-acting human insulin analogue such as

insulin detemir has been shown to result in more significant insulin resistance, oxidative stress, skeletal muscle ectopic fat accumulation and mitochondrial impairments compared to hyperglycaemia alone (Liu et al., 2009). Studies consistently demonstrate that physical activity is associated with reduced insulin requirements in people with type 1 diabetes, which would therefore have positive effects on insulin sensitivity and cardiovascular health (Ramalho et al., 2006, Yki-Jarvinen et al., 1984). Indeed, Muis et al. (2006) found in a cross-sectional study of 416 patients that increased physical activity was associated with lower daily insulin needs, while increased body weight and triglyceride levels were associated with higher insulin doses.

Skeletal muscle health is adversely affected in people with type 1 diabetes due to increased metabolic stress, vascular impairments and insulin resistance compared to non-diabetics (Coleman et al., 2015). Impaired skeletal muscle health may lead to a vicious cycle of insulin resistance, impaired glucose and lipid disposal, and reduced basal metabolic rate, which would affect an individual's ability to manage their type 1 diabetes. In addition, impaired insulin-stimulated vasodilation can reduce blood flow and therefore glucose delivery for uptake into skeletal muscle (Makimattila et al., 1996, Baron et al., 1991). It is believed that maintaining or improving skeletal muscle health in individuals with type 1 diabetes, potentially through exercise, can contribute to delaying complications.

People with type 1 diabetes have higher IMTG content than weight and activity matched non-diabetics, similar to type 2 diabetic individuals, which is associated with impaired insulin sensitivity (Perseghin et al., 2003, Dube et al., 2006). It is assumed that as IMTG deposition increases, lipotoxicity ensues (van Herpen and Schrauwen-Hinderling, 2008), enhancing stress on the tissue. The chronic hyperglycaemic state may be a relevant mechanism of exaggerated IMTG accumulation in those with type 1 diabetes as Perseghin et al. (2003) found a relationship between IMTG and HbA1c. When Perseghin et al. (2003) separated

type 1 participants into good (HbA1c <7.5%) and poor (HbA1c >7.6%) metabolic control, those with good control showed higher insulin-stimulated glucose metabolic clearance rate in association with lower IMTG, suggesting that when good glucose homeostasis is achieved these abnormalities may be at least partially reversed. As exercise training is associated with greater IMTG turnover in people without type 1 diabetes, exercise may improve insulin sensitivity through similar mechanisms in people with type 1 diabetes.

Increased inflammation and oxidative stress is another characteristic of type 1 diabetes due to the excessive plasma glucose (Brownlee, 2005, Coleman et al., 2015, Russell et al., 2009). Oxidative stress likely contributes to diabetic myopathy through upregulation of atrophy-related genes (atrogenes) atrogin-1 and MuRF-1 resulting in lower muscle mass (Mastrocola et al., 2008). In addition, the high oxidative stress can impair the transcription of glucose transporters which contributes to the development of insulin resistance (Bloch-Damti and Bashan, 2005). A single bout of exercise elicits anti-inflammatory effects and exercise training may decrease basal levels of the pro-inflammatory cytokine IL-6 in people with type 1 diabetes (Fischer, 2006). The anti-inflammatory effects of exercise may also have a beneficial effect on β cell mass due to marked increases in circulating growth hormone, IGF-1, GLP-1 and IL-1 receptor agonist. For people recently diagnosed with type 1 diabetes, exercise training may have numerous positive effects that would reduce daily insulin needs and possibly preserve β cell function. Animal studies suggest that exercise preserves β cell mass (Coskun et al., 2004) and insulin secretion per β cell islet (Huang et al., 2011), possibly due to anti-inflammatory effects of exercise. Preservation of β cell mass is clinically significant as it reduces the risk of retinopathy and neuropathy and reduces the incidence of hypoglycaemia (Narendran et al., 2015).

The majority of the evidence used to create the physical activity guidelines (Colberg et al., 2016) applied to people with type 1 diabetes are based on studies on

healthy individuals and those with type 2 diabetes (Chimen et al., 2012). These are clearly different conditions, so it is important to establish the effects of exercise in people with type 1 diabetes in order to establish the most effective strategies. The number of exercise training studies conducted in people with type 1 diabetes is relatively small; however, it appears that so long as the appropriate precautions are made regarding blood glucose levels during and around exercise, people with type 1 diabetes should take part in regular exercise (Riddell et al., 2017). Supervised exercise training has been shown to improve $\dot{V}O_{2max}$ (Laaksonen et al., 2000, Yki-Jarvinen et al., 1984), blood lipid profile (Laaksonen et al., 2000) and endothelial function (Fuchsjager-Mayrl et al., 2002). Regular exercise training has consistently been shown to reduce insulin requirements between 6 and 15% indicating improved insulin sensitivity (Ramalho et al., 2006, Fuchsjager-Mayrl et al., 2002, Yki-Jarvinen et al., 1984); however, studies investigating the effects of physical activity on glycaemic control in people with type 1 diabetes are less clear (Chimen et al., 2012). A number of factors may account for the difficulty in detecting a change in glycaemic control with training. For example, carbohydrate consumption often increases around the time of exercise in people with type 1 diabetes to reduce the risk of hypoglycaemia and this may counteract any improvement in glycaemia with training (Kennedy et al., 2013). Nonetheless, improvements in the above mentioned factors collectively reduce the risk of diabetic complications and increase life expectancy (Moy et al., 1993). Unfortunately, as discussed earlier, similarly to the rest of the UK population, many people with type 1 diabetes fail to reach the minimum physical activity guidelines (Brazeau et al., 2014, Bohn et al., 2015). The following sections will discuss the potential barriers that prevent such a large proportion of the general population from meeting the physical activity guidelines.

2.6. Barriers to Exercise

As outlined above, regular exercise training is associated with greater $\dot{V}O_{2max}$ and insulin sensitivity which reduce the risk of chronic disease and mortality (Booth et al., 2017, Myers, 2003). However, the majority of the worldwide population fail to reach the physical activity guidelines of 150 minutes of accumulated moderate intensity or 75 minutes of vigorous intensity exercise per week. Lack of time is cited as the main barrier to exercise (Trost et al., 2002) as many individuals feel that increasing work hours and family commitments mean they cannot achieve the physical activity guidelines. Other common barriers within the general population include limited access to facilities and appropriate equipment, difficulty with transportation, inadequate financial resources and lack of motivation (Trost et al., 2002).

Additional exercise barriers have been identified in overweight individuals either at high risk or already with type 2 diabetes (Korkiakangas et al., 2009). Physically active adults have been shown to experience fewer exercise barriers compared with those leading more sedentary lives and adults of normal weight experience fewer barriers than overweight adults (Kowal and Fortier, 2007). The feeling of being too fat or embarrassed to exercise are common additional barriers among overweight adults, that tend to be higher in women than men (Ball et al., 2000). However, in obese individuals, increases in weekly exercise and/or weight loss are associated with reduced barriers (Korkiakangas et al., 2009). This suggests that starting and maintaining an exercise programme for a sustained period (~3 months) may reduce barriers and help to maintain adherence. The population has to be taken into account when designing exercise training programmes to overcome the major barriers to exercise, as will be covered in **Chapter 4**. People with type 1 diabetes have additional, specific barriers to exercise that will be discussed below, and these are the focus of **Chapters 6, 7** and **8**.

2.6.1. Specific Exercise Barriers in People with Type 1 Diabetes

As with the rest of the population, people with type 1 diabetes are recommended to do regular exercise (Colberg et al., 2016, Riddell et al., 2017). However, many fail to achieve the exercise guidelines and many programmes designed to increase physical activity in people with type 1 diabetes have failed (Brazeau et al., 2014). In addition to the usual barriers cited by the general population such as lack of time, work commitments and cost (Brazeau et al., 2008, Jabbour et al., 2016, Lascar et al., 2014), additional barriers to exercise can exist for those with type 1 diabetes including fear of hypoglycaemia, loss of glycaemic control and inadequate knowledge around exercise management (Lascar et al., 2014).

Iatrogenic hypoglycaemia is defined in patients with type 1 diabetes as all episodes of abnormally low plasma glucose concentration that expose the individual to potential harm (Seaquist et al., 2013). A specific glycaemic threshold value that defines hypoglycaemia cannot be assigned, as the thresholds are lower after recent antecedent hypoglycaemia or higher in poorly controlled type 1 diabetic patients. However, the International Hypoglycaemia Study Group have defined Level 1 hypoglycaemia as a blood glucose concentration ≤3.9 mmol/L; Level 2 hypoglycaemia as ≤2.9 mmol/L which indicates serious, clinically important hypoglycaemia; and Level 3 severe hypoglycaemia as when the patient requires assistance from another individual to treat the hypoglycaemia (International Hypoglycaemia Study Group, 2017). The symptoms of hypoglycaemia range in seriousness from loss of coordination and mental confusion to convulsions, unconsciousness, brain damage and even death (Becker and Ryan, 2000, Cryer et al., 2003). At the very least, an episode of hypoglycaemia is a nuisance and a distraction, but in extreme cases can be fatal; hypoglycaemia accounts for 2-4% of deaths in people with type 1 diabetes (Cryer et al., 2009). People with type 1 diabetes suffer on average two episodes of symptomatic hypoglycaemia per week

(although studies using continuous glucose monitors show higher unnoticed incidences; (Kubiak et al., 2004)) and one severe, temporarily-disabling hypoglycaemic episode per year (MacLeod et al., 1993, Gubitosi-Klug et al., 2017).

Hypoglycaemia is associated with cardiovascular, neurologic and psychological morbidities (Gill et al., 2009, Oyer, 2013). During hypoglycaemia there is a large autonomic stimulus which promotes haemodynamic changes and increases workload on the heart, which may have dangerous consequences in some patients. Hypoglycaemia also stimulates an increased pro-inflammatory cytokine response (Razavi Nematollahi et al., 2009), leading to elevated plasma viscosity because of an increase in erythrocyte concentration and coagulation, and potentially affecting plaque stability (Frier et al., 2011). These changes may promote intravascular coagulation and thrombosis, and encourage development of tissue ischaemia. Hypoglycaemic episodes also prolong cardiac repolarisation and can increase the risk of cardiac arrhythmias and sudden death (Frier et al., 2011, Cryer, 2011). The longer term effects of repeated exposure to severe hypoglycaemia on cognitive function are less clear. Data from the diabetes control and compilations trial (DCCT) showed no differences in cognitive function between intensive treatment (strict avoidance of hypoglycaemia) and standard treatment arms over 20 years (Frier, 2011). However, severe hypoglycaemia in children with type 1 diabetes has been associated with poorer cognitive development (Bjorgaas, 2012).

Fear of hypoglycaemia is a major barrier to exercise in people with type 1 diabetes (Lascar et al., 2014, Brazeau et al., 2008). Exercise generally increases the risk of hypoglycaemia in people with type 1 diabetes due to their inability to decrease endogenous circulating insulin, increases in exercise-induced insulin sensitivity and an exercise-induced mobilisation of insulin from the site of administration (Wasserman and Zinman, 1994). The current exercise guidelines recommend that people with type 1 diabetes should accumulate 150 minutes of

moderate-intensity exercise per week (Colberg et al., 2016). However, as a bout of moderate-intensity exercise is associated with a rapid decline in glycaemia (-4.43 mmol/L h^{-1} on average; (Garcia-Garcia et al., 2015)), it is not surprising that many people with type 1 diabetes avoid exercise, especially if they have had a previous bad experience of exercise-related hypoglycaemia. However, because of their increased risk of macro-vascular disease, regular exercise should be encouraged as the overall cardio-metabolic benefits outweigh the immediate risks, provided certain precautions are taken (Codella et al., 2017, Riddell et al., 2017). Greater knowledge of the glucoregulatory responses to different types of exercise may enable precautions to be made to manage exogenous insulin and nutritional intake accordingly. This will be explored in **Chapters 6**, **7** and **8**.

Post-exercise, late-onset hypoglycaemia is also a common complaint and barrier to exercise for those with type 1 diabetes (MacDonald, 1987). The risk of hypoglycaemia has been shown to persist for up to 31 hours post exercise (MacDonald, 1987). Glucose requirements following moderate-intensity exercise exhibit a biphasic pattern with increases occurring both immediately post and 7-11 hours post-exercise (McMahon et al., 2007, Tsalikian et al., 2005). The immediate effects of exercise on glucose uptake are mediated by the residual effects of contraction-stimulated glucose uptake which are independent of insulin. Beyond the first few hours, the increased risk of hypoglycaemia is primarily due to increased insulin sensitivity which can vary according to duration and intensity of exercise that was performed (Mikines et al., 1988). The increased insulin sensitivity and continued extraction of glucose from the circulation has been suggested to be due to increased glycogen synthase activity to replenish muscle glycogen stores (Teich and Riddell, 2016). Deactivation of glycogen synthase kinase 3 is also thought to promote activation of glycogen synthase which will mean that glucose uptake is maintained (Cross et al., 1995). However, enhanced GLUT4 translocation and microvascular

perfusion are also important for the changes in glucose uptake. Special care may be required to prevent post-exercise hypoglycaemia after afternoon or evening exercise because there is a greater risk of nocturnal hypoglycaemia (Gomez et al., 2015). The risk of nocturnal hypoglycaemia following 45 minutes of moderate-intensity exercise in the day has been shown to be as high as 30-40% (Tsalikian et al., 2005, Maran et al., 2010, Iscoe et al., 2006). For these reasons, continuous glucose monitoring is used in **Chapter 6** to assess the 24-hour glucose profiles in people with type 1 diabetes following exercise.

Hyperglycaemia is another potential barrier for people with type 1 diabetes as certain types of exercise can cause an increase in blood glucose (Riddell et al., 2017). High-intensity aerobic exercise tends to increase blood glucose concentrations because insulin levels do not rise in the portal circulation of someone with type 1 diabetes to compensate for the increase in catecholamines. In someone without type 1 diabetes, the increase in catecholamines and hyperglycaemia is compensated for by an increase in insulin secretion, usually at the end of the exercise session. Prolonged and severe hypoinsulinaemia (possibly due to missed insulin dose, removed insulin pump or illness) increases the risk of elevated circulating or urinary ketone bodies. In these situations, high intensity exercise can increase hyperglycaemia and lead to ketoacidosis. This is because in the absence of insulin, muscle cells may not be able to take up glucose to use as a fuel, so instead rely on fatty acids and ketones. Hyperglycaemia and ketoacidosis may cause dehydration and decrease blood pH resulting in impaired performance and severe illness. Rapid ketone production can lead to ketoacidotic abdominal pain and vomiting, and in some cases may require emergency assistance. Therefore, people with type 1 diabetes are recommended to delay an exercise session if blood glucose concentration is >14 mmol/L and blood or urinary ketones are elevated (Riddell et al., 2017, Narendran et al., 2017).

2.7. Using High-Intensity Interval Training to Overcome Barriers to Exercise

2.7.1. Brief history of High-Intensity Interval Training

After the Second World War, interval training became a widespread training method used by a number of European runners and was popularized by Emil Záptopek who won the 5,000 m, 10,000 m and marathon in the same Olympics. However, it wasn't until the 1960s that the first scientific studies on interval training were conducted by the Swedish physiologist Per Oløf Astrand, who developed long interval training at a velocity between critical velocity and $\dot{V}O_{2max}$ (Astrand et al., 1960). Shortly after, Christensen et al. (1960), from the same group, proposed very short interval training consisting of 10-second intervals at 100% $\dot{V}O_{2max}$ interspersed with 10 seconds of recovery. This paper, involving two well trained male runners, was the first published paper to describe the metabolic response during interval training. This paper described how the two participants were able to achieve high oxygen uptake values during the intervals with a low increase in blood lactate as they were able to compensate for the oxygen deficits of each work interval during the rest periods. Around 10 years later, Karlsson and Saltin (1971) took biopsies at various points throughout an interval training protocol in three recreationally trained men which demonstrated that phosphocreatine was progressively depleted after each interval. Later, in the 1970s, Eddy et al. (1977) conducted a 7-week training intervention where one group did interval training (one minute on, one minute off at 100% $\dot{V}O_{2max}$) and the other did steady state cycling (70% $\dot{V}O_{2max}$). This study showed identical changes in $\dot{V}O_{2max}$ following the 7 weeks. Together, this body of work provided the basis for the explosion in interval training-related research over the last 15 years.

Today, high-intensity interval training (HIT) is defined as brief, intermittent periods of vigorous exercise, interspersed with periods of rest or active recovery.

When energy expenditure is matched to traditional MICT, HIT can induce similar or even superior improvements in performance and cardio-metabolic parameters in healthy and diseased populations (Wisloff et al., 2007, Tjonna et al., 2008). Furthermore, there is evidence that HIT may be more enjoyable than continuous exercise (Bartlett et al., 2011, Jung et al., 2014), unless the intervals are too strenuous or difficult to complete, and levels of enjoyment appear to increase with chronic training (Heisz et al., 2016). Therefore, HIT may offer an effective, alternative training strategy to traditional continuous aerobic exercise which currently forms the basis of the physical activity guidelines (Colberg et al., 2016). The main HIT protocols investigated in the literature are aerobic interval training (AIT) (Wisloff et al., 2007), sprint interval training (SIT) (Burgomaster et al., 2005) and constant load low-volume HIT (Little et al., 2011).

The rationale for implementing AIT was derived from evidence that the interval design enables individual to exercise at higher intensities thereby challenging the pumping ability of the heart more than would be possible at lower intensities (Tjonna et al., 2008). Indeed, studies have shown that AIT induces superior benefits in $\dot{V}O_{2max}$ to MICT in a number of at-risk populations (Molmen-Hansen et al., 2012, Wisloff et al., 2007, Tjonna et al., 2008). A typical AIT session consists of 4 x 4 minute intervals of treadmill exercise at 90-95% of heart rate max (HR_{max}) interspersed with 4-minute recovery periods at 50-70% HR_{max}. Tjonna et al. (2008) found that 16 weeks of AIT improved endothelial function, insulin signalling in adipose tissue and skeletal muscle and blood pressure in metabolic syndrome patients. Wisloff et al. (2007) showed that 12 weeks of AIT induced superior cardiovascular effects to MICT in heart failure patients and Molmen-Hansen et al. (2012) found AIT to be effective in hypertensive patients. Although AIT is very effective even in these at-risk populations, it is still time consuming, as a typical session lasts 38 minutes including the warm up time. Furthermore, the exercise

intensity is very difficult to maintain throughout the session so requires high levels of motivation.

As discussed in Section 2.6., many people cite 'lack of time' as the major barrier to achieving the physical activity guidelines (Trost et al., 2002). In an attempt to overcome this barrier, SIT was developed as a time-efficient alternative to AIT and MICT (Gibala et al., 2012). Typical SIT sessions involve 4-6 repeated Wingate tests (30-second supra-maximal 'all-out' sprints) interspersed with 4 to 4.5 minutes of recovery. The higher intensity of exercise during SIT elicits greater metabolic signalling than moderate-intensity exercise resulting in increased turnover of ATP (Howlett et al., 1998). The greater activation of kinases including AMPK, p38 MAPK and CaMKII during SIT are associated with greater expression of PGC-1α mRNA, which is a major regulator of mitochondrial biogenesis (Egan et al., 2010). For a more detailed description of the training adaptations with SIT readers are referred to reviews by Gibala et al. (2012) and MacInnis and Gibala (2017). Just six sessions of SIT, totalling ~15 minutes of all-out cycling over two weeks, has been shown to increase skeletal muscle oxidative capacity (Burgomaster et al., 2005). Other interventions lasting 4-6 weeks have shown that SIT induces comparable increases in $\dot{V}O_{2max}$, insulin sensitivity, oxidative capacity, vascular function and microvascular adaptations in comparison to a typical MICT programme consisting of 40-60 minutes moderate-intensity training 5 times per week (Burgomaster et al., 2005, Burgomaster et al., 2008, Shepherd et al., 2013a, Cocks et al., 2013). SIT is therefore a time-efficient training strategy as there is a weekly time commitment of just 1.5 hours compared to 4.5 hours with MICT. Furthermore, with just 2-3 minutes spent exercising per session there is a low training volume.

Despite the effectiveness of SIT to stimulate metabolic adaptations, the supra-maximal Wingate-based protocol requires an 'all out' effort and therefore high levels of motivation to complete, and tend not to be well tolerated and as they often

cause nausea (Gibala et al., 2012). Furthermore, the total time commitment of a SIT session is still approximately 30 minutes because of the long rest periods, and performing Wingates requires expensive, specialised exercise equipment. As a result, Gibala's laboratory subsequently developed a constant load low-volume HIT protocol whereby participants perform repeated 60-second bouts of cycling at a constant workload equating to ~90% HR_{max} interspersed with 60 seconds of recovery (Little et al., 2011). The reduced intensity during each interval allows for a reduced rest period, resulting in an overall reduction in the time commitment per session. In a small pilot study, Little et al. (2011) showed that six HIT sessions over 2 weeks reduced average 24-hour blood glucose concentrations and post-prandial glucose excursions in people with type 2 diabetes. This mode of HIT has been shown to be effective at improving insulin sensitivity and GLUT4 protein content in lean males after just 2 weeks (Hood et al., 2011) and recently, Tan et al. (2018) found that 6 weeks of HIT three times per week increased microvascular density and oxidative capacity of type 1 and 2 muscle fibres in healthy sedentary young women. It therefore appears that constant load low-volume HIT provides a practical alternative to traditional MICT to improve physiological and metabolic health in both a healthy and a diseased population so will be the basis of all the studies in this thesis.

2.7.2. Introducing HIT into the 'Real World'

Although HIT is a time-efficient and effective means to improve cardio-metabolic health, exercise participation in the general population remains low and rates of obesity and type 2 diabetes continue to rise. Current evidence for the efficacy of HIT in comparison to traditional MICT originates from researcher led laboratory-based investigations involving relatively small participant numbers, on specialised equipment, under close supervision to ensure correct exercise intensities are

achieved (Gillen and Gibala, 2014, Weston et al., 2014). Whilst time-efficient, laboratory-based HIT does not address the other barriers to exercise including limited access to facilities and appropriate equipment, difficulty with transportation or inadequate financial resources (Korkiakangas et al., 2009). Furthermore, the feasibility of HIT has been questioned by public health researchers, citing the strenuous nature and high levels of motivation required to complete the exercises as barriers (Hardcastle et al., 2014, Biddle and Batterham, 2015, Courneya, 2010). Future studies should therefore focus on functional and practical forms of HIT that can be used in the 'real world'.

Two studies to date have attempted to apply HIT to a more ecologically valid environment (Shepherd et al., 2015, Lunt et al., 2014). Lunt et al. (2014) found modest improvements in cardiorespiratory fitness in a cohort of overweight/obese participants following a 12-week intervention of either AIT, MVIT (maximal volitional intensity training: repeated 30 second maximal walking or jogging up a slope interspersed with a 4-minute recovery period) or moderate intensity walking. The study was performed in a community park setting with the aim of making it more 'real world'. Following this, Shepherd et al. (2015) investigated HIT performed in a "real world" gym setting and found improved cardio-metabolic risk factors and psychological health in previously inactive adults. However, the latter intervention still relied on instructor led classes where participants were given verbal motivation throughout, which is not always available. These interventions also rely on exercise equipment and there is still the travel time needed to attend the sessions. An interesting finding from the study by Shepherd et al. (2015) was that adherence was greater in the HIT group than the MICT group, presumably because of the greater time commitment required in the MICT group (five sessions of 30-50 mins five times per week compared to three sessions of <25 mins for the HIT group), further supporting the use of HIT as a time efficient exercise strategy.

In an attempt to overcome the remaining barriers, home-based exercise interventions have been successfully introduced in various populations (Jaggers et al., 2013, Perri et al., 1997). Such studies based around traditional MICT have shown that home-based interventions can increase adherence, even over a supervised training programme (Perri et al., 1997). Therefore, low-volume HIT performed as a home-based intervention, using simple bodyweight exercises, may be particularly attractive as it would combine the time efficient nature of HIT with the ease and cost effectiveness of home-based interventions, minimising barriers to exercise. Recently, Blackwell et al. (2017) compared the efficacy of a 4-week unsupervised home-based HIT protocol to laboratory-based HIT in middle-aged individuals (Blackwell et al., 2017). The Home-HIT protocol consisted of 1 minute intervals involving three equipment-free exercises (star jumps, squat thrusts and static sprints) interspersed with 90 seconds of walking. Blackwell et al. (2017) reported that participants completed all training sessions in the home-based HIT group; however, this was only monitored using simple self-report diaries, which is not an objective measure. The use of HR monitors that would allow the research team to remotely monitor and record session completion rates, and that target training thresholds that are achievable with a completely unsupervised home-based training programme, has not been done. Furthermore, the study of Blackwell et al. (2017) was only 4 weeks in duration which may not be long enough to test the longer-term effectiveness of home-based HIT. Therefore, **Chapter 4** will investigate the effects of a 12-week home-based HIT intervention in a previously sedentary, obese population with elevated CVD risk to test whether this programme can effectively reduce barriers to exercise in this at-risk population.

2.7.3. HIT and People with Type 1 Diabetes

HIT may also have the potential to overcome the barrier of fear of hypoglycaemia in people with type 1 diabetes. A series of studies have shown that addition of a short sprint to a bout of moderate-intensity exercise can increase blood glucose concentration to provide a means to counter the fall in post exercise glycaemia (Fahey et al., 2012, Bussau et al., 2006, Bussau et al., 2007, Davey et al., 2013a). Bussau et al. (2006) investigated whether a short sprint can counter the rapid fall in glycaemia that occurs during moderate intensity exercise and decrease the risk of post exercise hypoglycaemia. Seven males with type 1 diabetes injected their normal insulin and ate their usual breakfast before performing a 20-minute cycle at 40% $\dot{V}O_{2max}$ followed immediately by a 10-second maximal sprint (sprint trial) or rest (control trial). The moderate-intensity exercise caused a significant drop in blood glucose in both trials. However, the 10-second sprint opposed a further fall in blood glucose for 120 minutes, whereas blood glucose fell by ~3.6 mmol/L in the control trial. The stabilisation of glycaemia following the sprint trial was associated with elevated catecholamines, growth hormone and cortisol. Therefore, HIT, which consists of high intensity bouts interspersed with low intensity recovery periods, may stabilise blood glucose and reduce the risk of hypoglycaemia both during exercise and in the post-exercise period. As such, HIT could lessen the fear of hypoglycaemia as a barrier to exercise in people with type 1 diabetes, as well as being a time-efficient and effective strategy for improving cardio-metabolic health.

2.8. Thesis Overview

The overarching aim of the thesis is to investigate the effects of practical forms of high-intensity interval training (HIT) in obese individuals with elevated cardiovascular disease (CVD) risk and people with type 1 diabetes. To realise this aim, a novel home-based HIT (Home-HIT) programme was developed with the intention of

reducing the major barriers to exercise. Five studies were subsequently conducted to examine the effects of HIT and Home-HIT in obese individuals with elevated CVD risk and people with type 1 diabetes.

Section 2.6. described the major barriers to exercise that prevent a large proportion of the population from meeting physical activity guidelines and Section 2.7.2. concluded that a home-based intervention based on the scientific principles of HIT represents a good strategy to increase exercise participation. **Chapter 4** investigated the effects of a novel Home-HIT intervention in obese individuals at elevated CVD risk designed to overcome these barriers. In **Chapter 4** we used the principle of the low-volume HIT protocol developed by Little et al. (2011) to create a new Home-HIT intervention tailored to obese individuals with low fitness and mobility. During the 12-week intervention a novel HR monitoring system was used to remotely monitor adherence and compliance (ability to meet target HR thresholds). Changes in $\dot{V}O_{2peak}$, insulin sensitivity, body composition, flow-mediated dilation and aortic pulse wave velocity were assessed to determine the physiological effectiveness of Home-HIT in comparison to two control groups (laboratory-based HIT and home-based MICT). In addition, muscle biopsies were taken to assess changes in mitochondrial density, IMTG content and GLUT4 protein expression using quantitative immunofluorescence microscopy as these are classical adaptations associated with $\dot{V}O_{2peak}$ and insulin sensitivity.

Section 2.3.1.1. outlined the importance of microvascular health for an optimal insulin-sensitive phenotype and the impairments that occur with obesity and physical inactivity. **Chapter 5** aimed to investigate whether Home-HIT was effective at improving microvascular density and enzymes controlling NO production and quenching, and whether these correlate with increases in $\dot{V}O_{2peak}$ and insulin sensitivity. To achieve this, immunofluorescence microscopy was used to assess the protein content and phosphorylation status of the enzymes within the endothelial

layer of the skeletal muscle microvasculature as well as change in capillary density following 12 weeks of Home-HIT, laboratory-based HIT and home-based MICT.

In **Chapters 6, 7** and **8** the focus shifts to people with type 1 diabetes as the aim was to develop exercise strategies that reduce the major exercise barriers of "lack of time" and fear of hypoglycaemia (Section 2.6.1.). **Chapter 6** aimed to investigate, for the first time, the effects of a single bout of HIT on risk of hypoglycaemia during exercise and in the subsequent 24-hour period in people with type 1 diabetes. To achieve this aim we examined the effects of a fasted bout of HIT and MICT in comparison to a control day with no exercise on 24-hour glucose levels using continuous glucose monitoring. Following this, **in Chapter 7**, we investigated whether 6 weeks of HIT improves markers of metabolic health, including $\dot{V}O_{2peak}$, glycaemic control, blood lipid profile and arterial stiffness in people with type 1 diabetes in comparison to MICT. During this six-week training period, blood glucose concentrations were monitored before and after all exercise sessions that were performed in the fed state to provide further evidence that HIT could reduce the acute risk of hypoglycaemia compared to MICT.

Finally, in **Chapter 8** we investigated whether the Home-HIT programme introduced in **Chapter 4** could remove the remaining exercise barriers in people with type 1 diabetes as an exercise strategy that is time-efficient, inexpensive, requires no equipment or travel time to "intimidating" gym environments and removes the barrier of fear of hypoglycaemia. In addition, to assessing the physiological effectiveness of Home-HIT, the participants that took part in the study in **Chapter 8** completed an online survey to qualitatively assess the effectiveness of Home-HIT. To date, no study has qualitatively explored the attitudes and barriers to exercise in people with type 1 diabetes in conjunction with an exercise programme specifically designed to remove barriers and increase exercise participation.

Over the course of the five studies, the aim was to provide evidence that practical HIT strategies can remove many of the major exercise barriers for obese individuals and people with type 1 diabetes that could potentially be used to improve population health by increasing physical activity participation. Secondly, this thesis aims to provide clear mechanistic evidence to explain the physiological effectiveness of Home-HIT as a means to reduce the risk of cardio-metabolic disease.

Chapter 3 General Methods

Chapter 3

This General Methods chapter serves to outline the methods that are common across the experimental chapters, as well as describing in detail the staining procedures for the immunofluorescence microscopy work in **Chapters 4** and **5**.

3.1. Incremental Exercise Test to Determine $\dot{V}O_{2peak}$ and W_{max}

Throughout this thesis, the incremental exercise tests to volitional exhaustion were performed on an electromagnetically braked cycle ergometer (Corival, Lode, Groningen, The Netherlands) to determine maximal aerobic power output (W_{max}) and $\dot{V}O_{2peak}$ using an online gas collection system (MOXUS modular oxygen uptake system, AEI technologies, Pittsburgh, PA). The test consisted of 3-minute stages starting at 25 W, and the workload was increased by 35 W at each stage until subjects could not maintain a cadence of >50 rpm. $\dot{V}O_{2peak}$ was taken as the highest value achieved over a 15-second recording period. Heart rate (HR) was measured throughout the tests using a Polar RS400 (Kempele, Finland) HR monitor.

3.2. Flow Mediated Dilation

Following 20 minutes supine rest, blood pressure measurements were made in triplicate using an automated sphygmomanometer (Dianamap; GE Pro 300V2, Tampa, Florida) on the contralateral arm. Endothelial function was measured using brachial artery flow-mediated dilation (FMD) in accordance with the current guidelines (Thijssen et al., 2011). Briefly, a 10 MHz multi-frequency linear probe attached to a high-resolution ultrasound machine (T3000, Terason, Burlington, MA) was used to image the brachial artery in upper arm and a rapid inflation/deflation pneumatic cuff was positioned on the imaged arm distal to the olecranon process. A one-minute baseline recording was made before the cuff was inflated to 220 mmHg for 5 minutes. The cuff was then deflated and the response monitored for 3 minutes.

The scans were performed by the same sonographer at each time point to reduce between scanner variation.

Post-test analysis of the brachial artery was performed using custom-designed edge-detection and wall-tracking software (Dicom Encoder), of which the reproducibility and validity have been demonstrated elsewhere (Woodman et al., 2001). This software tracks the vessel walls and blood velocity trace in B-mode frames via pixel density and frequency distribution algorithm. First, regions of interest (ROI) on every individual study were identified by the sonographer. The ROIs allowed automated calibration for diameters on the B-mode image and velocities on the Doppler strip. A ROI was then manually drawn around the optimal area of the B-mode image and within this. Baseline diameter, flow and shear were calculated using continuous edge detection and wall tracking software. The peak artery FMD was defined as the peak percentage change in artery diameter from baseline to during the 3 minutes post cuff release. The software automatically calculated the relative diameter change, time to peak (following cuff release) and shear rate area-under-the-curve (SR_{AUC}). Despite the initial region of interest selection being operator-determined, the remaining analysis was independent of operator bias.

3.3. Arterial Stiffness

Aortic pulse wave velocity (PWV) measurements were made in triplicate using a semi-automated device and software (SphygmoCor, AtCor Medical, Sydney, Australia). Carotid-femoral PWV measurements were performed to characterise the aortic stiffness in participants by placing a single high fidelity applanation tonometer at the proximal (carotid) and distal (femoral) pulse, to record sequentially over 10 waveforms. The QRS complex was measured simultaneously using an ECG. The pulse transit time was calculated by subtracting the time between the R wave of the

ECG and the foot of the proximal waveform from the time between the R wave and the foot of the distal waveform. To determine the distance used for PWV, an anthropometric tape was used to determine the distance from the carotid measurement site to the suprasternal notch subtracted from the distance between the femoral measure and suprasternal notch. The day-to-day variability measured as the coefficient of variation for aortic PWV in our laboratory is 4%.

Obesity and sedentary behaviour are associated with increased arterial stiffness, which in turn is associated with an increased risk of cardiovascular events and mortality (Laurent et al., 2001, Vlachopoulos et al., 2010). Degenerative changes that occur in the walls of large arteries are thought to contribute to increased stiffening over time. Large artery stiffness increases left ventricular afterload (Laurent et al., 2006) and is associated with left ventricular hypertrophy (Toprak et al., 2009). Furthermore, increased arterial stiffness is associated with hypertension (Laurent et al., 2006, Vlachopoulos et al., 2010, Vlachopoulos et al., 2006). Arterial stiffness is dependent on the dynamic and material properties of the artery (Zieman et al., 2005). For example, sympathetic activation of the vascular smooth muscle causes vasoconstriction, decreasing lumen diameter and increased arterial stiffness, and exercise training has been shown to improve autonomic control in obese women (Trombetta et al., 2003). Improved arterial stiffness with exercise training may also be due to improved endothelial function, a reduction in low-grade inflammation and a reduction in the wall-to-lumen ratio (Donley et al., 2014).

3.4. Blood Analysis

Insulin sensitivity was measured using the oral glucose tolerance test (OGTT) procedure, as described by Matsuda and DeFronzo (1999). A cannula was inserted into an antecubital vein and a baseline 10 ml blood sample was taken before

consumption of a 25% glucose beverage containing 75g of glucose and 225 ml of water. Further 5 ml blood samples were collected at 30, 60, 90 and 120 minutes after glucose ingestion. Plasma was separated by centrifugation (10 min at 3000 rpm at 4˚C) and stored at -80°C until analysis.

Plasma glucose concentrations at each time point, as well as fasting plasma cholesterol and triglyceride concentrations, were analysed using a Randox chemistry analyser (Randox RX Series, the RX Daytona™). Plasma insulin concentrations were determined by commercially available enzyme linked immuno-sorbent assay (ELISA) kit (Invitrogen, UK). Area under the curve (AUC) for insulin and glucose during the OGTT was calculated using the conventional trapezoid rule. Insulin sensitivity index (ISI) was calculated using the Matsuda index (Matsuda and DeFronzo, 1999) using the following equation:

$$\text{Matsuda ISI} = \frac{10{,}000}{\sqrt{(FPG \; x \; FPI) x \; (P\bar{G} \; x \; P\bar{I})}}$$

Where FPG = fasting plasma glucose concentration, FPI = fasting plasma insulin concentration, $P\bar{G}$ is mean plasma glucose concentration over 2 hour OGTT, $P\bar{I}$ is mean plasma insulin concentration.

3.5. Muscle Biopsies

Resting muscle biopsies were taken from the lateral portion of the *m. vastus lateralis*, approximately 25-50% of the distance from the lateral joint line and the greater trochanter under local anaesthesia (0.5% Marcaine), using the Weil-Blakesley conchotome technique as described previously (Baczynska et al., 2016). Excess blood and visible collagen or fat were removed before samples were embedded in Tissue-Tek OCT Compound (Sakura Finetek Europe, Zoeterwoude, Netherlands) and immediately frozen in liquid nitrogen cooled isopentane (Sigma-Aldrich, Dorset, UK). Samples were then stored at -80°C until analysis.

3.5.1. Quantitative Immunofluorescence

Details of the specific quantification techniques used can be found in Table 3.1. and all techniques have been previously validated (Shepherd et al., 2013b, Cocks et al., 2016, Bradley et al., 2014). GLUT4 content was assessed using the method described by (Bradley et al., 2014) which has previously been optimised for analysis of GLUT4 in human skeletal muscle using negative controls to confirm there were no problems with antibody cross reactivity (Bradley et al., 2014). All techniques used frozen muscle biopsy samples cryosectioned to a thickness of 5µm onto uncoated glass microscope slides so that transverse orientated samples could be used for analysis.

Fibres were either fixed in 3.7% formaldehyde (IMTG, mitochondria) or acetone and ethanol (GLUT4). Where sections were fixed with formaldehyde, this was followed by a brief 30-second rinse in dH_2O, followed by permeabilisation using 0.5% triton X-100. Following three 5-minute washes with phosphate-buffered saline (PBS, 137 mmol/L sodium chloride, 3 mmol/L potassium chloride, 8 mmol/L sodium phosphate dibasic, 3 mmol/L potassium phosphate monobasic) sections were incubated overnight with appropriate primary antibodies. On day 2, slides were washed 3 times for 5 minutes in PBS before secondary antibodies were applied to sections for 1 hour at room temperature. Mitochondria and GLUT4 slides were washed a final 3 times in PBS before being mounted with Mowiol and a coverslip. IMTG slides were moved to a dark room after the secondary antibody incubation where they were incubated a final time in BODIPY for 20 minutes, followed by 3 x 5 minute PBS washes before being mounted using Vectashield (Sigma-Aldrich, UK).

Table 3.1. Staining protocol for GLUT4, mitochondria and intramuscular triglycerides

Treatment	GLUT4	Mitochondria	IMTG
Fixation	Acetone:ethanol (3:1) for 5 mins	Formaldehyde 3.7% for 1 hour	Formaldehyde 3.7% for 1 hour
Washes	3 x 5 min PBS washes	3 x 30 second dH_2O washes	3 x 30 second dH_2O washes
Permeabilisation		0.5% Triton X-100 for 5 mins	0.5% Triton X-100 for 5 mins
Washes	3 x 5 min PBS washes	3 x 5 min PBS washes	3 x 5 min PBS washes
Incubation	Primary antibodies overnight Dystrophin (1:400); MHCI (1:100); GLUT4 (1:200)	Primary antibodies 1 hour Dystrophin (1:400); MHCI (1:100); COX IV (1:100)	Primary antibodies 45 mins Laminin (1:50); MHCI (1:100)
Washes		3 x 5 min PBS washes	3 x 5 min PBS washes
Incubation	Secondary antibodies 1 hour GAMIgM 546 (1:100); GAMIgG2b 633 (1:100); GARIgG 488 (1:200)	Secondary antibodies 1 hour GAMIgM 546 (1:250); GAMIgG2b 633 (1:100); GAMIgG2a 488 (1:250)	Secondary antibodies 30 mins GAMIgM 546 (1:200); GARIgG 633 (1:200)
Washes	3 x 5 min PBS washes	3 x 5 min PBS washes	3 x 5 min PBS washes Move to dark room
Incubation			BODIPY for 20 mins (1:200)
Mounting	Mowiol	Mowiol	Vectashield

MHCI = myosin heavy chain I; PBS = phosphate-buffered saline; COX IV = cytochrome c oxidase

Capillarisation

Sections were fixed in acetone and ethanol (3:1) for 5 minutes before 3 x 5 minute PBS washes. Muscle sections were incubated overnight with anti-myosin type I (developed by Dr Blau DSHB). On day 2, sections were washed 3 times for 5 minutes with PBS before incubation with UEA-I-FITC (Sigma-Aldrich) and WGA-633 as markers of the endothelium and plasma membrane, respectively for 1 hour. Lastly, sections were washed 3 more times with PBS for 5 mins before being mounted using Mowiol.

Vascular Enzymes

NOX2 and $p47^{phox}$ protein content in the skeletal muscle microvascular endothelium and sarcolemma were assessed using the previously developed immunofluorescence staining protocol and quantification technique (Cocks et al., 2012, Cocks et al., 2013), adapted to allow for differentiation between capillaries and terminal arterioles (Cocks et al., 2016). Capillary and terminal arteriole specific eNOS content and eNOS ser^{1177} phosphorylation were also assessed using previously established methods (Cocks et al., 2016); however, the method was adapted to allow for assessment of individual vessel eNOS ser^{1177}/eNOS ratio to be calculated.

First, sections were fixed in acetone and ethanol (3:1) for 5 minutes. For assessment of eNOS ser^{1177}/eNOS ratio, sections were triple stained with antibodies against eNOS (Transduction Laboratories, Lexington, KY, USA), p-eNOS ser^{1177} (Cell Signalling Technology, Beverly, MA, USA) and anti-α smooth muscle actin (αSMA; Abcam, Cambridge, UK). For assessment of NOX2 and $p47^{phox}$ content sections were double stained with either NOX2 or $p47^{phox}$ (kind gift from Prof Mark Quinn, Montana State University) and anti-αSMA. All sections were then incubated with appropriate secondary antibodies (Invitrogen, Paisley, UK) in combination with the endothelial marker *Ulex* Europaeus-FITC conjugated (UEA-I-FITC; Sigma-

Aldrich, UK). A plasma membrane marker, wheat germ agglutinin-633 (WGA-633; Invitrogen, Paisley, UK), was also included when staining samples for NOX2 and $p47^{phox}$. Between each incubation with antibodies, sections received 3 x 5 minute PBS washes. For image capture, sections were mounted using Mowiol. See Table 3.2. for general staining protocol for the vascular enzymes.

Table 3.2. Staining protocol for vascular enzymes

Treatment	
Fixation	Acetone:ethanol (3:1) for 5 mins
Washes	3 x 5 min PBS washes
Incubation	Primary antibodies overnight
Washes	3 x 5 min PBS washes
Incubation	Secondary antibodies 1 hour
Washes	3 x 5 min PBS washes
Mounting	Mowiol

PBS = phosphate-buffered saline

3.5.2. Image Capture

Images for mitochondrial density and capillarisation were acquired using a Lecia DM6000FS widefield microscope and 40x 0.6 numerical aperture objective. Images for GLUT4 and IMTG content were acquired using an inverted confocal microscope (Zeiss LSM-710, Carl Zeiss, Germany) with a 63x oil immersion objective and the images for the vascular enzymes were captured using a 40x oil immersion objective. Alexa Fluor 405 was excited using the 405 nm line of the diode laser and detected

with 371-422 nm emission. FITC fluorescence was excited with a 488nm multiline argon laser and detected with 493–557nm emission. DAPI fluorescence was excited with a 405nm diode laser and detected with 410-495nm emission. The images were acquired at a resolution of 1,024 X 1,024 pixels and stored in 24-bit tagged image format file format. No image processing was carried out prior to intensity analysis and identical settings were used for all image capture for each variable within each participant.

3.5.3. Image Analysis

All image analysis was performed using ImagePro Plus 5.1 (Media Cybernetics Inc, Bethesda, MD, USA).

Mitochondrial density

Mitochondrial density was assessed using the method described by Shepherd et al. (2013b). Briefly, fluorescence intensity of the mitochondrial stain was quantified by measuring the signal intensity within the intracellular regions of a mask created by the dystrophin-633 stain, in a fibre type specific manner.

GLUT4 Content

GLUT4 fluorescence intensity was quantified by measuring the signal intensity within the intracellular regions of a mask created by the dystrophin-633 stain in a fibre type specific manner determined by the MHCI stain. For each subject at least 10 type I fibre images and 10 type II fibre images were captured per section pre and post training. Therefore, for each subject at least 20 images (10 type I fibres and 10 type II fibres) were analysed for both pre- and post-training.

IMTG Analysis

Fibre type specific IMTG analysis to assess peripheral and central regions of the myocyte was assessed using the method described in (Shepherd et al., 2017). This method was adapted in order to assess IMTG content, lipid droplet size and number in the peripheral and central regions of the myocyte. The peripheral region was defined as the 5μm below the plasma membrane. Briefly, an intensity threshold was uniformly selected to represent a positive signal for IMTG. IMTG content was expressed as the positively stained area fraction relative to the total area of each muscle fibre. IMTG density was calculated as the number of IMTG objects relative to area. The mean area of individual IMTG (lipid droplets) objects was used as a measure of lipid droplet size.

Capillarisation

Capillaries were quantified in a fibre type specific manner manually, using the UEA-I, WGA-633 and myosin heavy chain images. The following indexes were measured (Hepple et al., 1997): 1) the number of capillaries around a fibre (capillary contacts), 2) capillary-to-fibre ratio on an individual fibre basis and 3) capillary-fibre perimeter exchange (CFPE) index. In addition, overall capillary density was determined. Quantification of capillarisation was performed only on transverse fibres. In line with previous studies assessing capillarisation, at least 50 complete fibres were included in each analysis (Porter et al., 2002). Fibre cross-sectional area and perimeter were measured on calibrated images using ImagePro Plus 5.1 software.

The indexes used to quantify capillarity include global indexes include capillary density and capillary-to-fibre ratio, while the individual fibre indexes include capillary contacts, capillary-fibre perimeter exchange and fibre perimeter per capillary (Latroche et al., 2015). The global indexes provide a global indication of

muscle blood supply but they assume regular distribution of capillaries and do not take fibre size and type into account. The individual fibre indexes allow evaluation of capillarity at the level of the individual myofibre. The individual indexes also provide more information on capillarity specific to fibre size and type, which will be important in **Chapter 5** to provide information on adaptations to Home-HIT. Capillary density provides information on the number of capillaries per unit area; however, it gives little information on the capillary supply to individual fibres and is dependent on fibre size changes. Capillary-to-fibre ratio gives information on the number of fibres per unit area. However, there is little information on capillary supply to individual fibres. Capillary contacts provides information on the number of capillaries in contact with each fibre but without information on the effects of fibre size.

Vascular Enzymes

Blood vessels were divided into either capillaries or arterioles using the αSMA image. The endothelial (UEA-I-FITC) outline was then overlaid onto the corresponding vascular enzyme image. Fluorescence intensity of the vascular enzyme signal was then quantified within the endothelial specific area. Diameter of the arterioles was determined on calibrated images. Vessels larger than 20 μm in diameter were excluded to remove 3rd and 4th order arterioles (Wu et al., 2011) from the analysis, which rarely appear in muscle cross-sections. As eNOS and eNOS ser^{1177} phosphorylation had been stained on the same sections it was possible to establish eNOS ser^{1177}/eNOS ratio on an individual vessel basis, as the same endothelial outline could be placed over both eNOS and eNOS ser^{1177} images. Cell membrane specific fluorescence for NOX2 and $p47^{phox}$ was determined using the WGA-633 stain to create an outline of the cell membrane. This mask was then

overlaid onto the corresponding image to determine membrane specific fluorescence intensity for NOX2 or $P47^{phox}$.

Chapter 4 A Proof of Principle Study to Determine Adherence and Compliance to a 12-Week Home-Based High-Intensity Interval Training Programme and the Effects on Markers of Cardio-Metabolic Health in Obese Individuals with Elevated Cardiovascular Disease Risk

Chapter 4

4.1. ABSTRACT

Objectives: To investigate the efficacy of a novel home-based high-intensity interval training (Home-HIT) intervention in obese individuals, with elevated cardiovascular disease (CVD) risk. It was hypothesised that Home-HIT would 1) have high adherence to the prescribed exercise intensity (compliance), 2) improve markers of CVD risk, and 3) lead to favourable skeletal muscle adaptations.

Methods: Thirty-two obese adults with at least two additional CVD risk factors (age 36±2y; BMI 34.3±0.8kg·m^{-2}), completed one of three 12-week interventions: Home-HIT (*n*=9), laboratory-based supervised HIT (Lab-HIT; *n*=10) or home-based moderate-intensity continuous training (Home-MICT; *n*=13). Adherence and compliance were monitored online using a heart rate monitor and mobile app. Changes in $\dot{V}O_{2peak}$, insulin sensitivity, body composition, flow-mediated dilation (FMD) and aortic pulse wave velocity (PWV) were assessed. As classical training adaptation markers muscle mitochondrial density, intramuscular triglyceride (IMTG) content and GLUT4 protein expression were assessed.

Results: Adherence and compliance did not differ between groups (P>0.05). Training increased $\dot{V}O_{2peak}$, Matsuda insulin sensitivity index and FMD (P<0.05). BMI, body fat percentage, visceral fat mass and aortic PWV all decreased (P<0.05). Training also increased muscle mitochondrial density, IMTG content and GLUT4 protein expression (P<0.05). Between group differences were not significant for any of the variables.

Conclusions: Despite having no supervision during exercise, Home-HIT had a high adherence at the prescribed exercise intensity, comparable to fully supervised Lab-HIT, resulting in improved cardio-metabolic health. This study provides strong evidence that Home-HIT is an effective strategy to remove barriers to exercise and improve health in obese individuals with elevated CVD risk.

4.2. INTRODUCTION

Despite overwhelming evidence that an inactive lifestyle leads to chronic disease and premature death (Booth et al., 2012), many people fail to meet public health physical activity guidelines (Earnest, 2009, Hallal et al., 2012). Part of the reason for the current apathy is that recommendations (at least 150 minutes of moderate-intensity exercise per week) are difficult to attain for many, with “lack of time” the most commonly cited barrier (Trost et al., 2002). High-intensity interval training (HIT), involving repeated bouts of high-intensity exercise interspersed with periods of recovery, has been proposed as a time-efficient and effective strategy to improve cardio-metabolic health (Currie et al., 2013, Little et al., 2011, Hood et al., 2011, Gillen and Gibala, 2014). However, the applicability of current HIT programmes to the sedentary obese population has been disputed by public health experts (Biddle and Batterham, 2015, Courneya, 2010, Hardcastle et al., 2014), who cite the strenuous nature and complex protocols as major barriers in sedentary, exercise-naïve individuals. Furthermore, most successful HIT interventions to date have been laboratory-based investigations under optimal conditions with continuous supervision and using specialised equipment (Little et al., 2011, Hood et al., 2011, Tjonna et al., 2008). This creates additional barriers to exercise including difficulties with access to facilities (including travel distance and cost) and embarrassment due to negative body image (Korkiakangas et al., 2009).

To eliminate many of the aforementioned barriers to exercise we developed a HIT intervention tailored to exercise-naïve individuals with low fitness and mobility. The programme used simple ‘on the spot’ movements using one's own body weight, creating an intervention that could be performed in the participant’s home without supervision or equipment. The following pilot study aimed to investigate the efficacy of this novel home-based HIT (Home-HIT) intervention in previously sedentary obese individuals, with elevated cardiovascular disease (CVD) risk. To achieve this aim, participants completed 12 weeks of Home-HIT and the following were investigated: 1)

adherence and compliance (defined as ability to meet prescribed heart rates), 2) changes in cardio-metabolic health markers, and 3) the change in classical markers of training adaptation within skeletal muscle. Two control groups were included to assess the effectiveness of Home-HIT. A fully supervised laboratory-based HIT (Lab-HIT) group was employed to assess the effect of Home-HIT compared to HIT performed under optimal conditions. Secondly, a home-based moderate-intensity continuous training (Home-MICT) group was used to assess the effect of Home-HIT compared to a group advised to meet the recommended physical activity guidelines (Colberg et al., 2016). It was hypothesised that Home-HIT would 1) have high adherence at the prescribed exercise intensity (compliance), 2) improve markers of cardiovascular and metabolic health, and 3) lead to favourable skeletal muscle adaptations.

4.3. METHODS

Participants

Thirty-two sedentary obese adults (BMI >30 kg·m^2 or waist/hip ratio of >0.9 in men and >0.85 in women) with at least 2 further CVD risk factors, according to the American Heart Association criteria (Grundy et al., 1999), completed the study (for participant characteristics see Table 4.1.). Participants were allocated to one of three 12-week exercise training groups: Home-HIT (n = 9); Home-MICT (n = 13); or Lab-HIT (n = 10) matched for age, BMI and $\dot{V}O_{2peak}$. Participants were free of diagnosed CVD and other contraindications to participate in an exercise intervention. All participants provided written informed consent, and the study was approved by the Black Country NHS Research Ethics Committee (West Midlands, UK) and conformed to the *Declaration of Helsinki*.

Table 4.1. Participant characteristics and overview of the number of patients that met American Heart Association coronary heart disease risk factor thresholds

	Home-HIT	Home-MICT	Lab-HIT
Age (yrs)	32 ± 3	38 ± 3	37 ± 2
Sex (male/female)	4/5	4/9	5/5
Height (cm)	168 ± 4	172 ± 3	172 ± 2
BMI ($kg \cdot min^{-2}$)	35.9 ± 1.4	33.3 ± 1.4	34.2 ± 1.3
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	25.9 ± 0.8	26.4 ± 1.8	24.8 ± 2.0
$\dot{V}O_{2peak}$ (L/min^{-1})	2.4 ± 0.2	2.5 ± 0.2	2.5 ± 0.2
Medication	1/9	8/13	4/10
Smoker/previous smoker	1/9	3/13	5/10
Family history	5/9	6/13	4/10
Obesity	9/9	13/13	10/10
Sedentary lifestyle	9/9	13/13	10/10
Impaired fasting glucose	1/9	1/13	1/10
Dyslipidaemia	9/9	12/13	7/10
Hypertension	4/9	7/13	1/10
Mean number of risk factors per participant	4 ± 0	4 ± 0	4 ± 0
Range of risk factors	3-5	3-6	3-6

Medication included blood pressure medication (e.g. ramipril, felodipine, losartan, amilodipine, indipamide), metformin or statins. Family history included diabetes and/or cardiovascular disease in an immediate family member. Obesity was classified as a BMI >30 $kg \cdot m^2$ or waist/hip ratio of >0.9 in men and >0.85 in women. Dyslipidaemia was defined as total cholesterol >11.1 $mmol.L^{-1}$, HDL <2.2 $mmol.L^{-1}$ or LDL >7.2 $mmol.L^{-1}$. Hypertension was classified as >140/90 mmHg or on antihypertensive medication and impaired fasting glucose was defined as fasting blood glucose >6.1 $mmol.L^{-1}$. Sedentary lifestyle was defined as persons not participating in a regular exercise programme or accumulating 30 minutes or more of moderate physical activity on most days of the week. Data are presented as mean±SEM when appropriate.

Pre-Training Testing

Participants performed an incremental exercise test to volitional exhaustion on an electromagnetically-braked cycle ergometer (Corival, Lode, Groningen, The Netherlands) to determine maximal aerobic power output (W_{max}) and $\dot{V}O_{2peak}$, using an online gas collection system (MOXUS, AEI technologies, Pittsburgh, PA) as described in **Chapter 3**. Waist-to-hip ratio was recorded and body composition was analysed using Dual-energy X-ray Absorptiometry (DXA). Finally, participants were provided with a physical activity monitor (ActiGraph GT3X+, Fort Walton Beach, FL) and diet diary so that habitual physical activity levels and diet could be assessed over 7 and 3 days, respectively.

Three to seven days after the incremental exercise test participants attended the laboratory after an overnight fast for pre-training testing. Vascular measures, muscle biopsies and oral glucose tolerance tests were undertaken. Participants were instructed to abstain from caffeine, alcohol and vigorous exercise the day before testing.

Vascular Measures

Flow mediated dilation (FMD), resting blood pressure and arterial pulse wave velocity (PWV) were measured as described in **Chapter 3**.

Muscle Biopsies

A resting muscle biopsy was taken from the lateral portion of the *m. vastus lateralis* under local anaesthesia (0.5% Marcaine), using the Weil-Blakesley conchotome technique (Baczynska et al., 2016). Samples were embedded in Tissue-Tek OCT Compound (Sakura Finetek Europe, Zoeterwoude, Netherlands) and immediately

frozen in liquid nitrogen cooled isopentane (Sigma-Aldrich, Dorset, UK). See **Chapter 3** for more information.

Blood Analysis and Oral Glucose Tolerance Test

Insulin sensitivity was measured using an oral glucose tolerance test (OGTT) as described in **Chapter 3**.

Week 4 Testing

A testing session took place during week 4 of the training programme instead of the 12th training session. Participants attended the laboratory following an overnight fast. FMD and arterial stiffness were measured, followed by assessment of $\dot{V}O_{2peak}$. Procedures were identical to pre-training testing.

Post-Training Testing

Post-training assessment of $\dot{V}O_{2peak}$ was performed instead of the 35th training session in the final week of training. Following the test all participants were provided with a physical activity monitor and diet diary. ~72h following the final training session post-training testing was conducted with procedures, methods and timings identical in all respects to the pre-training testing protocol.

Training Protocols

Training programmes started ~72h after pre-training testing. Participants trained for 12 weeks in one of three groups:

1. Home-based HIT (Home-HIT): repeated 1-minute bouts of exercise interspersed with 1 minute of rest. Participants were advised to achieve ≥80% of their predicted heart rate maximum (HR_{max}; 220–age) during the intervals. The 1-minute intervals were composed of two different sequential 30-second body weight exercises with no rest in between. Participants were provided with 9 exercise pairs, detailed in an exercise pack, and were free to choose which pairs of exercises they completed during each session (see Appendix). During weeks 1-4 participants were advised to complete 4 intervals, this then increased by one session each fortnight up to 8 during weeks 11-12.

2. Home-based MICT (Home-MICT): participants performed continuous exercise of their choosing (swimming, cycling or walking/running). Participants were advised to exercise at ~65% of predicted HR_{max} throughout the sessions. During weeks 1-4 participants were asked to exercise for 30 minutes which increased by 5 minutes each fortnight up to 50 minutes during weeks 11-12.

3. Laboratory-based HIT (Lab-HIT): participants performed repeated 1 minute bouts of exercise on a cycle ergometer (Excalibur Sport V2.0, Lode, Groningen, The Netherlands), interspersed with 1 minute of rest. During the intervals, participants exercised at an intensity of 100% W_{max} (Little et al., 2011) in order to elicit a HR of ≥80% HR_{max}. The number of intervals was identical to the Home-HIT group.

Participants in the Lab-HIT group attended the School of Sport and Exercise Sciences at Liverpool John Moores University (LJMU) to train 3x/week for 12 weeks. Participants in this group were excluded if ≥80% of sessions were not completed. To ensure adherence to training, the Lab-HIT sessions were scheduled at set times and

if participants did not attend a session, it was rescheduled for later that week. All the training sessions were supervised and participants were given strong verbal encouragement to ensure they met target HRs. Exercise intensity was monitored using a HR monitor (POLAR RS400, Kempele, Finland).

The home-based interventions were completed by the participants in an unsupervised place of their choosing outside of LJMU. To monitor training compliance and exercise intensity achieved, participants were given a POLAR H7 HR monitor and POLAR Beat mobile application (www.polar.com/beat/uk-en; Figure 4.1.). Following each training session HR was automatically uploaded to a cloud storage site (www.flow.polar.com), which could be accessed by the researchers to check adherence and that the correct HR threshold was being achieved during each session (training compliance). Participants were aware that the research team were monitoring their adherence and compliance. They were advised to train 3x/week, but this was not enforced. In contrast to Lab-HIT, participants in the home-based groups were responsible for scheduling their own training sessions, with no input from the research team. Participants were contacted by text/email once every two weeks to enquire about progress and any general issues around training. If participants were observed to have missed consecutive training sessions their email enquired as to whether there was a specific reason, however no direct encouragement was given for them to re-engage with training.

Figure 4.1. Methods used to monitor adherence and training intensity

A) Participants were provided with a Polar H7 heart rate (HR) monitor and mobile phone application Polar Beat. B) HR data from each session was downloaded to Polar Flow, a cloud based storage application so that adherence and compliance could be remotely recorded by the researchers. C) HR traces were analysed to provide information on adherence to the prescribed workload.

Quantitative Immunofluorescence

Quantitative immunofluorescence microscopy was used to investigate the effects of training on GLUT4 protein content, IMTG and mitochondria density. Details of the specific quantitative measurement methods and image analysis are described in detail in **Chapter 3**.

Statistical Analysis

The primary outcome variable was $\dot{V}O_{2peak}$. Previous research in our group (Shepherd et al., 2015, Shepherd et al., 2013a) has suggested an SD of 2.7-3.2 $ml \cdot kg^{-1} \cdot min^{-1}$ for the training response. Using this data 7-9 participants would be required to detect a clinically relevant within group difference in $\dot{V}O_{2peak}$ of 3.5 $ml \cdot kg^{-1} \cdot min^{-1}$ (Myers, 2003) with 80% power at a significance level of 5%. Differences in adherence and compliance between the three training protocols were assessed using a one-way ANOVA. Measures taken pre, mid and post-training were analysed using a two-way mixed design ANOVA with the within group factor 'training status' (pre vs. mid vs. post) and the between group factor 'training group' (Home-HIT vs. Home-MICT vs. Lab-HIT). Original data is presented for FMD as the same findings were reported when baseline diameter was analysed using allometric scaling (Atkinson and Batterham, 2013). Mitochondrial density, IMTG content, and GLUT4 protein expression were analysed using a three-way mixed ANOVA, with the between-group factor being 'training group' and within-group factors 'training status' (pre vs. post) and 'fibre type' (type I vs. type II). In the case of a significant interaction a Bonferroni post-hoc test was applied to locate the differences. Eight muscle biopsies were taken and analysed pre- and post-training in each group. Aortic PWV was recorded in 8 Home-HIT, 6 Home-MICT and 9 Lab-HIT participants due to difficulty scanning some participants. Matsuda Index values are reported for 9 Home-HIT, 10 Home-MICT and 9 Lab-HIT participants as it was not possible to obtain blood from all participants. All analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Significance was set at $P \leq 0.05$ and data are presented as mean ± SEM.

4.4. RESULTS

Adherence and Compliance to Training Protocols

Although not significant, training adherence tended to be different between the groups (*P*=0.053; Figure 4.2A). Post-hoc analysis revealed a trend for greater adherence to Lab-HIT compared to Home-MICT (*P*=0.081), but no significant differences were observed between Home-HIT and Lab-HIT (*P*=1.000) or Home-MICT (*P*=0.195). Training compliance, defined as the ability to meet target HR thresholds, showed no differences between the groups (*P*= 0.420; Figure 4.2B).

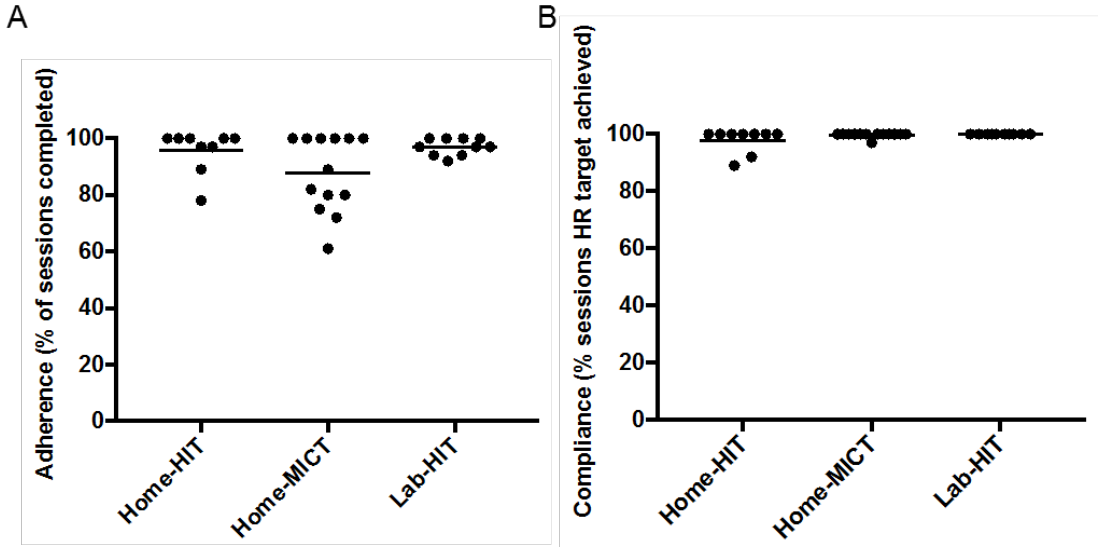

Figure 4.2. Adherence and compliance to home-based high intensity interval training (Home-HIT), home-based moderate intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT).

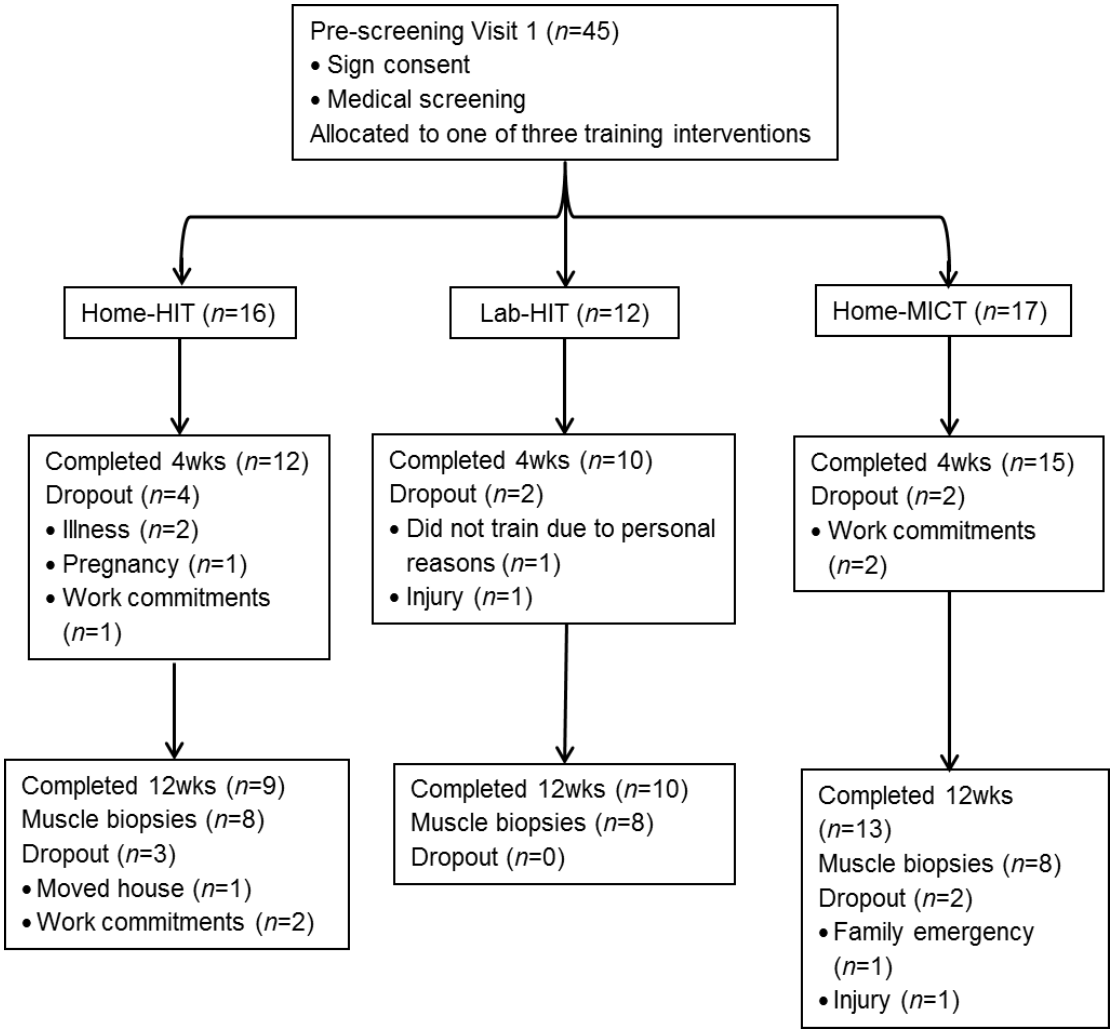

Figure 4.3. Flow chart of study design

General Characteristics

At baseline there were no differences in age, BMI or $\dot{V}O_{2peak}$ between groups (*P*=0.369; 0.455 and 0.898, respectively). Training increased $\dot{V}O_{2peak}$ (main effect of training, *P*<0.001), but no difference between groups was observed. Post-hoc analysis revealed that $\dot{V}O_{2peak}$ increased following 4 weeks of training compared to pre-testing (Home-HIT 9%, Home-MICT 6%, Lab-HIT 8%; *P*<0.001) and continued to increase further after 12 weeks compared to 4 weeks (Home-HIT 16%, Home-MICT 12%, Lab-HIT 20%; *P*<0.001). Training decreased body mass and BMI (main effect of training, *P*=0.003 and *P*=0.005, respectively), with no differences between groups. Post-hoc analysis revealed both body mass and BMI decreased following 4 weeks of training (*P*=0.004 and *P*=0.007, respectively). Although body mass and BMI were still significantly lower than pre-testing at 12 weeks (*P*=0.014 and *P*=0.022) no further reduction in either measure was observed compared to 4 weeks (*P*=0.439 and *P*=0.515, respectively). There was a 4% decrease in body fat percentage in all three groups (main effect, *P*<0.01), with no difference between groups (*P*=0.468). Visceral fat mass was also significantly reduced in all three groups (Home-HIT -27%; Home-MICT -12%; Lab-HIT -3%; main effect, *P*=0.025), with no difference between groups (*P*=0.304). There was no change in lean mass following training (*P*>0.05).

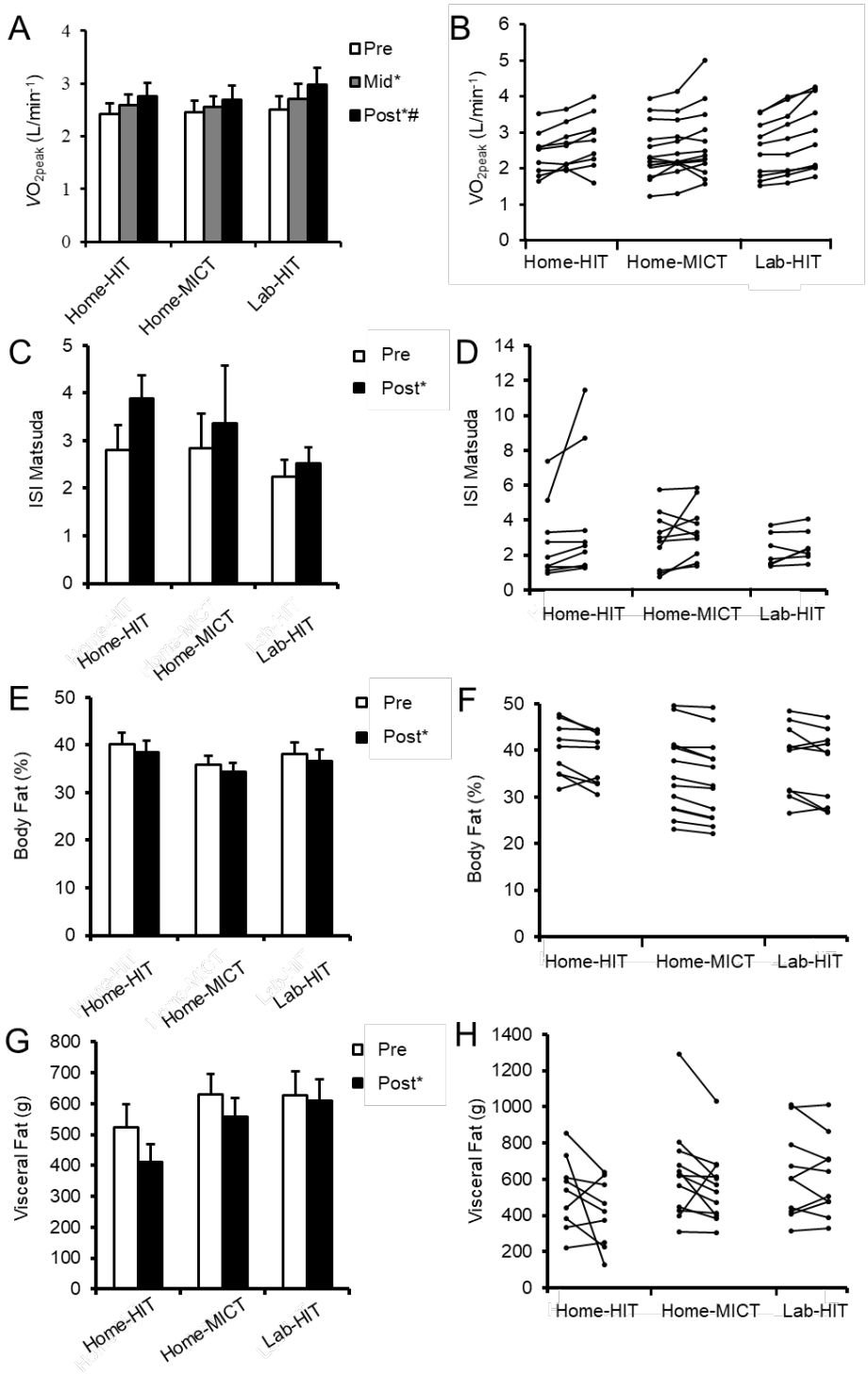

Figure 4.4. Effect of Home-based high-intensity interval training (Home-HIT), home-based moderate intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on $\dot{V}O_{2peak}$ (A), Matsuda Index (C), body fat percentage (E) and visceral fat (G).

B, D, F and H show the individual responses. *Indicates a significant difference from baseline (P<0.05) and #indicates a significant difference from week 4 (P<0.05). There were no significant differences in any of the variables between the groups. Data are presented as mean ± SEM.

Blood Variables

Insulin AUC decreased with training (Home-HIT -24%, Home-MICT -20%, Lab-HIT -18%; main effect, $P<0.001$) but there was no change in glucose AUC ($P>0.05$), with no difference between groups for either variable. The Matsuda ISI was significantly increased by 12 weeks of training (Home-HIT 39%, Home-MICT 8%, Lab-HIT 13%; main effect, $P=0.033$), with no difference between groups ($P=0.609$). There was no change in fasting plasma glucose, cholesterol, triglycerides, HDL or LDL (main effect, $P>0.05$). Data are presented in Table 4.2.

Vascular Measures

Baseline artery diameter was unchanged by training ($P=0.334$). There was a significant increase in FMD (main effect, $P<0.001$), with no difference between groups ($P=0.246$). Post-hoc analysis revealed that following 4 weeks of training FMD was not different from pre-training ($P=1.000$). However, FMD was significantly increased in all three groups following 12 weeks of training compared to pre-training (Home-HIT 30%, Home-MICT 43%, Lab-HIT 49%; $P<0.001$). Similarly, there was a significant effect of training on aortic PWV (main effect, $P<0.001$), but no difference between groups ($P=0.417$). Following post-hoc analysis there was no change in PWV following 4 weeks of training ($P=1.000$), but there was a significant decrease following 12 weeks of training compared to pre-training (Home-HIT -17%, Home-MICT -14%, Lab-HIT -4%; $P=0.04$). There was no difference in any of the blood pressure variables following training ($P>0.05$).

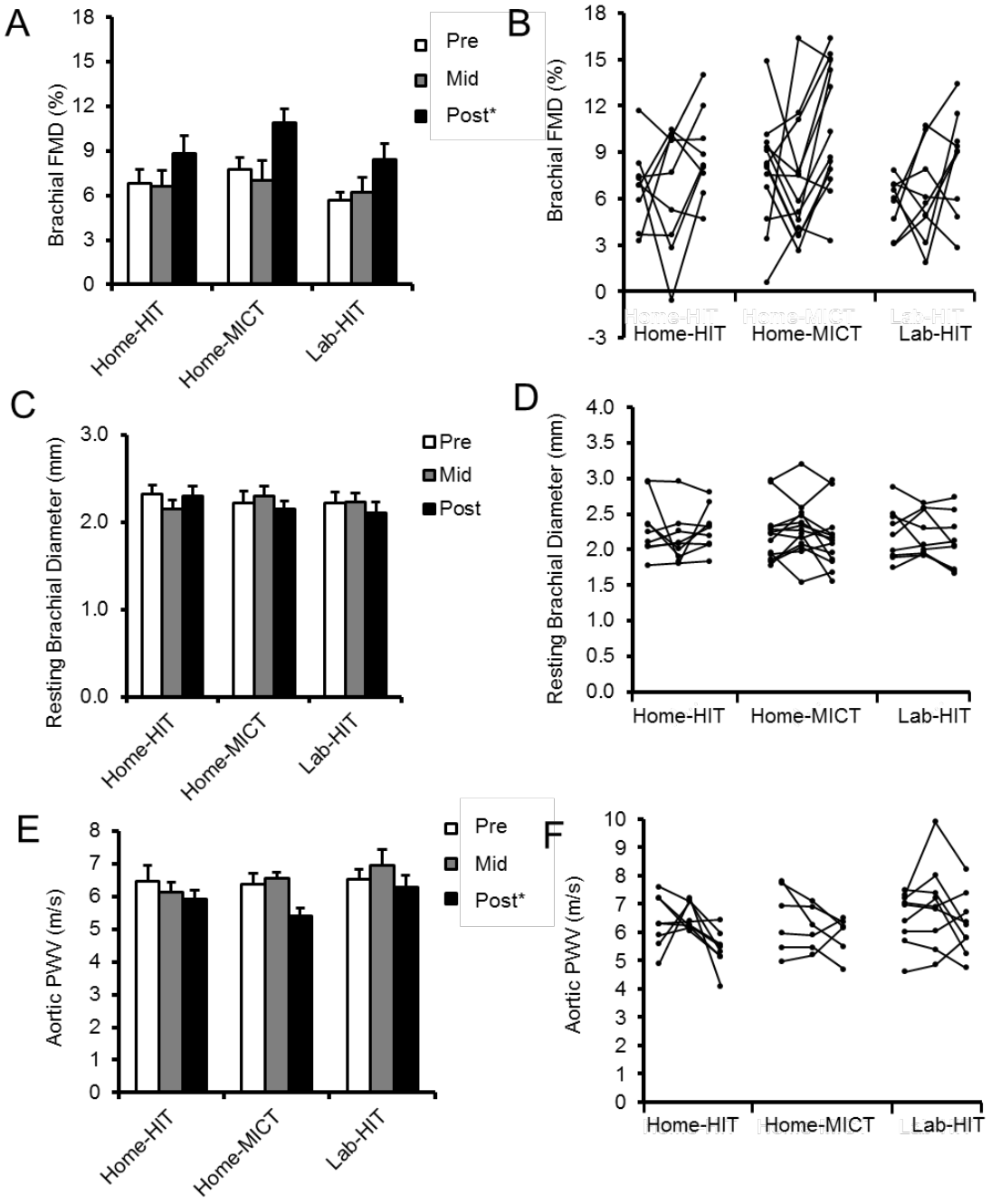

Figure 4.5. Effect of Home-based high-intensity interval training (Home-HIT), home-based moderate-intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on brachial artery flow mediated dilation (FMD) (A), resting brachial artery diameter (B) and aortic pulse wave velocity (PWV; C).

B, D, F and H show the individual responses. *Indicates significant difference from baseline (P<0.05).

Muscle Adaptations

COX IV protein expression (fluorescence intensity), a marker of mitochondrial density, was greater in type I fibres than type II fibres (main effect of fibre type $P<0.001$). Mitochondrial density increased in both type I (Home-HIT 14%, Home-MICT 6%, Lab-HIT 22%) and type II fibres (Home-HIT 34%, Home-MICT 11%, Lab-HIT 33%) following training (main effect; $P<0.001$), with no difference between groups.

GLUT4 content was not different between fibre types (main effect of fibre type, $P=0.221$). GLUT4 content was increased by training (main effect of training, $P=0.005$), with no difference between groups. There was also a strong trend towards a significant training*fibre type interaction ($P=0.061$), although not significant this trend was explored further. This analysis revealed that training increased GLUT4 content in type 2 fibres ($P=0.005$), but not type 1 fibres ($P=0.089$). Post-training GLUT4 content was higher in type 2 fibres than type 1 fibres ($P=0.02$), but not different pre-training ($P=0.983$).

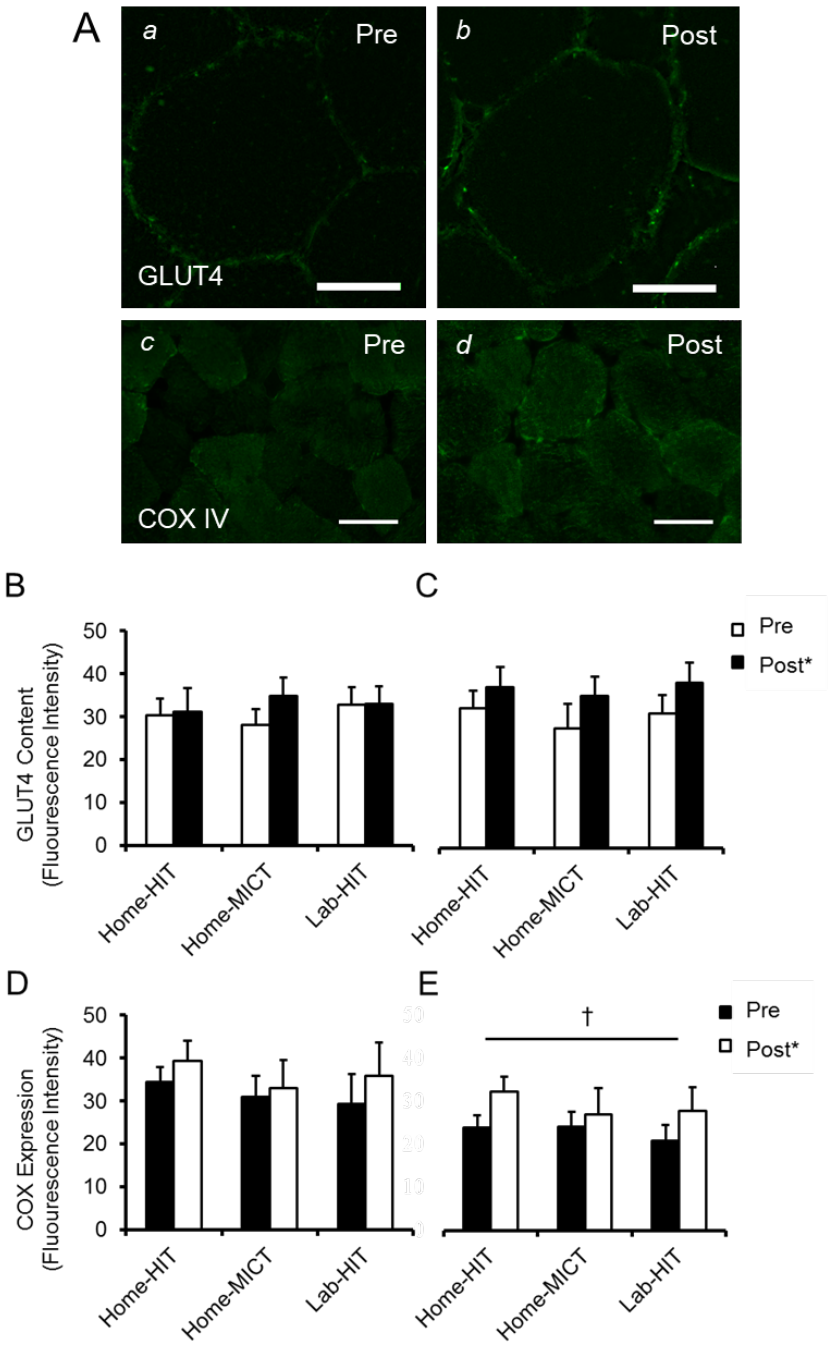

Figure 4.6. Effect of 12 weeks of home-based high-intensity interval training (Home-HIT), home-based moderate-intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on mitochondria density, indicated by COX IV fluorescence intensity, and GLUT4 content.

A) Representative confocal microscopy images of skeletal muscle GLUT4 fluorescence intensity pre (a) and post-training (b) and widefield microscopy images of COX IV fluorescence intensity pre (c) and post-training (d). Change in GLUT4 pre-post training is shown in type 1 fibres (B) and type 2 fibres (C). Change in COX expression pre-post training is shown in type 1 fibres (D) and type 2 fibres (E). *Indicates main effect of training (P<0.05); white bar = 50 µm. †Indicates a main effect of fibre type (P<0.05).

Total IMTG content was significantly greater in type 1 fibres than type 2 fibres (P<0.001) at baseline. Total IMTG content increased following 12 weeks of training (main effect, P=0.006), with no difference between groups. Region specific investigation of IMTG content (expressed as percentage area stained) revealed that central and peripheral IMTG content were also significantly greater in type 1 fibres than type 2 fibres at baseline (P<0.001). Central IMTG content increased following 12 weeks of training (main effect, P=0.026) and there was a non-significant trend towards an increase in peripheral IMTG content (P=0.06). The increase in total IMTG content was due to an increase in IMTG density in the central region (P=0.034) following training. IMTG size was unchanged by training (P=1.000).

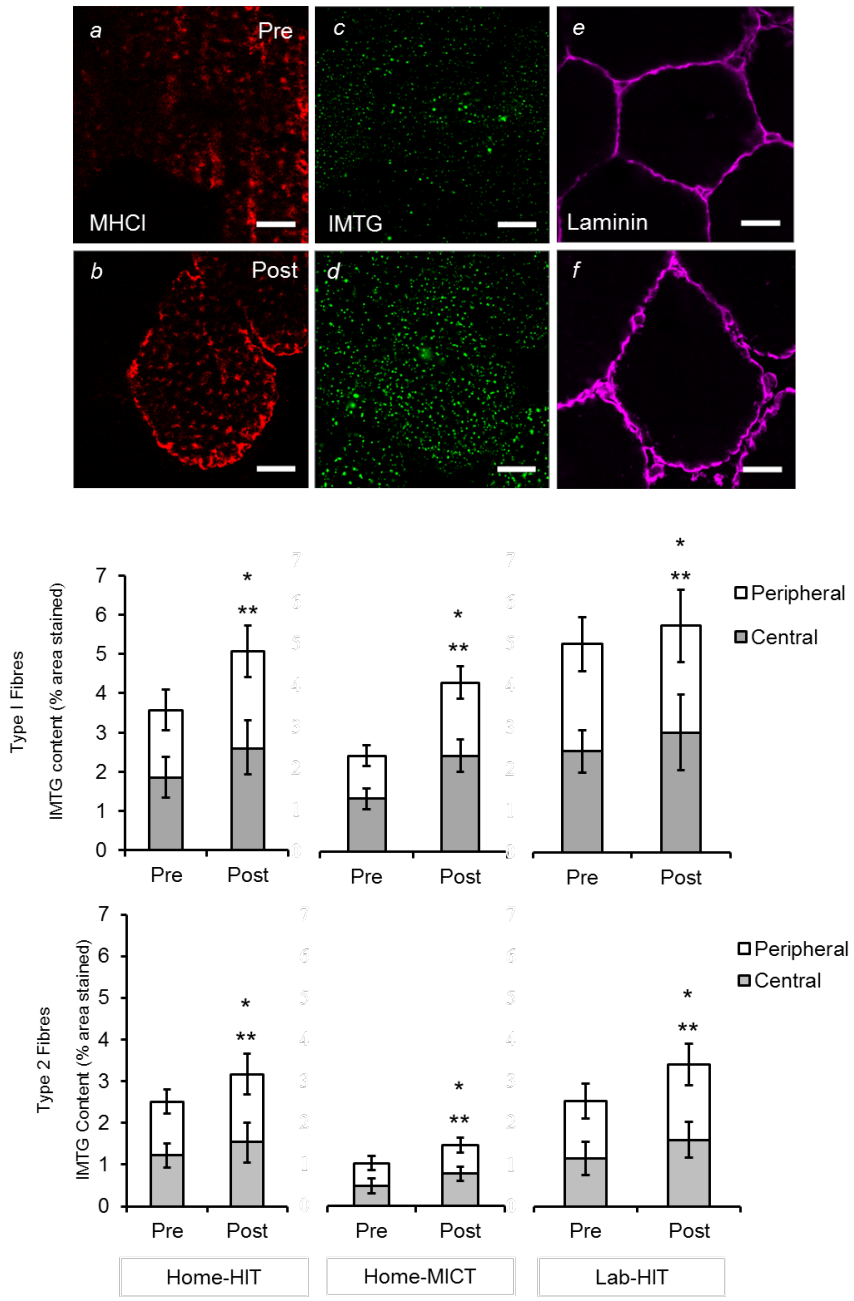

Figure 4.7. Effect of 12 weeks of home-based high-intensity interval training (Home-HIT), home-based moderate-intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on fibre type specific intramuscular triglyceride (IMTG) content.

(A) Representative confocal microscope images of myosin heavy chain I fibre type stain (red), IMTG (green) and plasma membrane using laminin (purple) pre (*a, c, e*) and post (*b, d, f*) training. Analysis was performed in both the peripheral (5μm border from the plasma membrane) and central (remainder of the cell) regions of each fibre. White bar = 20 μm. B and C show change in IMTG content pre and post training in type 1 and type 2 fibres in the central and peripheral region of the cells. *Indicates main effect of training in total IMTG content; **indicates a change in central IMTG content (P<0.05).

Table 4.2. Participant characteristics at pre, week 4 and post training

	Home-HIT			Home-MICT			Lab-HIT		
	Pre	Week 4	Post	Pre	Week 4	Post	Pre	Week 4	Post
Body mass (kg)	101.5±7.2	100.2±7.3*	100.1±7.5*	98.3±4.4	96.7 ± 4.5*	95.5±4.6*	101.1±4.8	99.6±4.4*	99.2±4.6*
BMI ($m \cdot kg^2$)	35.9±1.4	35.4±1.4*	35.4±1.5*	33.3±1.4	32.8 ± 1.4*	32.3±1.4*	34.2±1.3	33.7±1.1*	33.6±1.1*
Body fat (%)	40.1±1.9	-	38.4±1.9*	35.8±2.3	-	34.4±2.4*	38.1±2.4	-	36.6±2.5*
Visceral fat (g)	523.1±66.3	-	447.4±56.5*	645.4±69.6	-	557.2±53.3*	626.7±76.9	-	611.0±68.4*
Lean mass (kg)	56.7±4.8	-	58.1±5.0	57.9±2.6	-	57.5±2.9	58.1±4.0	-	58.8±4.0
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	23.8±0.8	25.9±0.8*	27.6±1.6*#	24.9±1.9	26.4±1.8*	28.0±2.3*#	24.8±2.0	26.9±2.2*	29.8±2.6*#
$\dot{V}O_{2peak}$(L/min^{-1})	2.4 ± 0.2	2.6 ± 0.2*	2.8 ± 0.3*#	2.5 ± 0.2	2.5 ± 0.2*	2.7 ± 0.3*#	2.5 ± 0.2	2.7 ± 0.2*	3.0 ± 0.3*#
W_{max} (W)	180±11	202±12*	213±11*#	182±15	188±15*	208±17*#	174±14	198±17*	221±16*#
W/H ratio	0.93±0.04	0.92±0.04	0.92±0.05	0.92±0.03	0.92±0.03	0.92±0.02	0.94±0.03	0.92±0.03	0.91±0.03
ISI Matsuda	2.8±0.7	-	3.9±1.2*	3.2±0.5	-	3.3±0.5*	2.3±0.4	-	2.5±0.3*
Glucose AUC ($mmol.L^{-1}.120min^{-1}$)	15551±521	-	15155±915	13979±969	-	14868±1474	18401±1351	-	17840±1255
Insulin AUC ($mmol.L^{-1}.120min^{-1}$)	13740±2583	-	10442±1839*	12556±2119	-	10043±1797*#	11914±1498	-	9723±1049*
Fasting glucose ($mmol.L^{-1}$)	5.4±0.2	-	5.0±0.3	5.2±0.2	-	5.5±0.3	5.2±0.2	-	5.6±0.2

Cholesterol ($mmol.L^{-1}$)	4.2±0.3	-	4.1±0.3	4.4±0.2	-	4.5±0.2	5.3±0.4	-	5.3±0.3
Triglycerides ($mmol.L^{-1}$)	1.0±0.1	-	1.0±0.2	1.1±0.2	-	1.3±0.2	1.6±0.3	-	1.4±0.2
HDL ($mmol.L^{-1}$)	0.8±0.1	-	0.9±0.1	1.0±0.1	-	1.0±0.1	1.1±0.1	-	1.1±0.1
LDL ($mmol.L^{-1}$)	3.7±0.3	-	3.7±0.3	3.7±0.4	-	3.8±0.3	4.4±0.5	-	4.4±0.5
MAP (mmHg)	86±3	82±3	83±3	86±2	90±3	85±2	91±3	87±1	90±3
SBP (mmHg)	119±4	115±4	115±4	127±5	124±4	125±4	122±3	123±2	121±3
DBP (mmHg)	70±3	66±3	66±3	73±3	73±3	72±3	68±2	69±1	67±3
Calorie intake (kcal)	1838±214	-	2216±196	1952±157	-	2043±112	1849±105	-	1696±150
Energy expenditure (kcal)	447±73	-	499±79	471±51	-	538±77	304±52	-	339±70

Values are means ± SEM. #Denotes a significant differences with training from baseline and *indicates a difference between week 4 and 12 ($P<0.05$). At baseline there were no differences in age, BMI or $\dot{V}O_{2peak}$ between groups ($P>0.05$).

4.5. DISCUSSION

This is the first study to successfully implement an unsupervised Home-HIT intervention in sedentary obese individuals with elevated CVD risk. Participants in the Home-HIT group had high adherence, which was similar to fully supervised Lab-HIT. Furthermore, the Home-HIT participants were able to meet the prescribed HR thresholds despite being completely unsupervised during exercise. All three exercise modes produced comparable improvements in markers of cardio-metabolic disease risk. Finally, the immunofluorescence microscopy data revealed skeletal muscle adaptations typically observed following endurance exercise training in all three groups. These pilot data provide strong evidence that our novel Home-HIT intervention is an effective strategy that removes many of the major barriers to exercise, and therefore may be an effective public health intervention for the sedentary obese population.

An Effective Training Programme with High Adherence and Compliance

A number of groups have shown HIT to be an effective training intervention (Cocks et al., 2013, Cocks et al., 2016, Little et al., 2011, Hood et al., 2011). However, to date most of the data has come from highly controlled laboratory-based studies (Weston et al., 2014), or field-based work with high levels of participant supervision (Shepherd et al., 2015, Lunt et al., 2014, Ong et al., 2009). As such, public health researchers have argued that although effective under optimal controlled conditions, HIT cannot become an effective public health intervention, when targeted at sedentary, exercise-naïve populations most in need (Biddle and Batterham, 2015, Courneya, 2010, Hardcastle et al., 2014). Here, we report high adherence rates (96%) to a novel Home-HIT programme in a sedentary obese group with elevated CVD risk, despite training sessions being unsupervised and self-scheduled at home.

Adherence to Home-HIT was in fact higher than two recent studies investigating supervised field-based HIT interventions (Lunt et al., 2014, Shepherd et al., 2015). Indeed, we (Shepherd et al., 2015) previously reported 83% adherence to an instructor-led HIT class in sedentary but otherwise healthy individuals over a 10-week programme, and in another study adherence to supervised outdoor sprint interval training (SIT) and aerobic interval training for 12 weeks was 75% and 55%, respectively, in overweight inactive adults (Lunt et al., 2014).

Moreover, despite previous concerns (Frazao et al., 2016), our data suggests obese participants with elevated CVD risk were able to exercise at sufficiently-high exercise intensities to elicit the benefits of HIT without close supervision. The Home-HIT intervention was designed for individuals with low fitness and mobility. Creating a personalised exercise service tailored to the user's needs is important as ability and health status has been shown to be a critical factor in maximising adherence to exercise (Morgan et al., 2016). One way in which current fitness level and mobility were taken into account was in the design of the exercises which ranged from, simple low-impact exercises to complex movements with higher impact. This allowed participants to modify exercise sessions, choosing exercises which elicited the desired HR response, but were suitable for their level of mobility and fitness. Unlike previous low-volume HIT studies (Little et al., 2011, Tan et al., 2018), the current protocol increased the number of intervals as participants progressed through the 12-week intervention, from 4 to 8 intervals. This may have influenced adherence to the programme as sedentary individuals have been shown to report pleasant feelings during the first 3-4 bouts of low-volume HIT, with the affective responses becoming more unpleasant with a higher number of intervals (Frazao et al., 2016). Importantly, enjoyment of HIT has been shown to increase with training (Heisz et al., 2016).

The Home-HIT protocol was designed to overcome the major barriers reported to prevent many people exercising as it was performed within the participant's home without any equipment. As such, significant barriers to exercise uptake and adherence like "intimidating" gym environments, difficulties with access to facilities, travel time and financial constraints (Trost et al., 2002, Morgan et al., 2016) were eliminated. Finally, the Home-HIT intervention was time-efficient. Training sessions lasted between 8 and 16 minutes and weekly time commitment in the Home-HIT group was 24-48 minutes compared to 90-150 minutes in the Home-MICT group. Moreover, as the exercise could be performed at home, further time was likely saved by not having to travel to the gym or other exercise locations.

A major strength of this study was the use of remote HR monitoring and mobile app to provide an objective measure of adherence and compliance. This is the first home-based investigation to use this technology, as other studies tend to rely on self-report (Blackwell et al., 2017, Halse et al., 2014). The novel exercise monitoring system may have positively influenced the findings of the current study, contributing to the high adherence observed in both home-based HIT (96%) and MICT (88%). Whilst home-based exercise programmes have a number of benefits, lack of support and supervision from exercise professionals may present a significant barrier. Indeed, a recent meta-analysis of barriers and facilitators to adherence in exercise referral schemes suggested that supervision from exercise professionals was needed to build and maintain motivation to exercise, and that lack of supervision induced negative opinions which are likely to reduce future adherence (Morgan et al., 2016). In the current study participants were aware that the research team was monitoring their adherence and received regular personalised emails (once every 2 weeks) asking how training was progressing. Although these emails did not explicitly instruct participants to train they may have led to a supportive environment (Morgan et al., 2016), contributing to the high adherence and compliance observed in both

home-based groups which were higher than previous field-based exercise interventions (Shepherd et al., 2015, Lunt et al., 2014). Importantly, such monitoring systems may provide a relatively inexpensive (approx. £40 per HR monitor and app) strategy to engage with participants and improve uptake, adherence, compliance and ultimately health outcomes.

Home-based HIT Effectively Improved Cardio-Metabolic Disease Risk Factors

Aerobic Capacity

There were similar improvements in $\dot{V}O_{2peak}$ in all three training groups despite the weekly time commitment in the Home-HIT group being 66-102 minutes less than Home-MICT, and having no supervision or encouragement as in the Lab-HIT group. The 16% increase in $\dot{V}O_{2peak}$ after 12 weeks of Home-HIT is similar to that reported in two recent meta-analyses investigating laboratory-based HIT in patients with lifestyle-induced cardio-metabolic disease (Weston et al., 2014) and SIT in sedentary individuals (Sloth et al., 2013). The findings from these pilot data have clinical importance given that training induced improvement in $\dot{V}O_{2peak}$ is associated with reduced risk of all-cause mortality (Lee et al., 2010). Moreover, our increase of 3.8 $ml \cdot kg^{-1} \cdot min^{-1}$ (~1 MET) after 12 weeks of Home-HIT is reported to be associated with a clinically meaningful 8-17% reduction in all-cause mortality[24].

$\dot{V}O_{2peak}$ was increased significantly by 9% following 4 weeks of Home-HIT, suggesting that despite having fewer intervals than previous low-volume HIT studies (Little et al., 2011) (4 vs 10), the intervention induces meaningful improvements that are similar to previous low-volume HIT interventions of similar duration (Hood et al., 2011, Gillen et al., 2012). Moreover, $\dot{V}O_{2peak}$ continued to increase by a further 7% between weeks 4 and 12 in the Home-HIT group, in line with the 6% increase in the MICT group and 12% increase in the Lab-HIT group. This is only the second study to

report physiological adaptations at two time points during a HIT intervention, supporting the previous work (Gillen et al., 2016) evidencing that $\dot{V}O_{2peak}$ does not plateau for at least 12 weeks of HIT.

Vascular Measures

On average, FMD increased by 2% after 12 weeks of Home-HIT. Brachial artery FMD is an independent predictor of CVD risk (Gokce et al., 2002, Green et al., 2011) and is a surrogate of coronary artery endothelial function (Anderson et al., 1995). Indeed, there is a 9% decrease in the future risk of cardiovascular events with each 1% increase in FMD (Green et al., 2011). Improved FMD has been suggested to be the result of elevated nitric oxide (NO) bioavailability following training (McAllister and Laughlin, 2006).

This is the first study to investigate the time course of FMD response with training in obese individuals with elevated CVD risk. Studies in healthy young volunteers have demonstrated that endothelial function (measured using FMD) is increased following 2-4 weeks of training, and that function is normalised after prolonged training (>6 weeks) due to structural adaptation i.e. increased brachial artery diameter (Green et al., 2017). The current results differ from this paradigm as increased endothelial function was observed only after 12 weeks, and there was no suggestion of arterial remodelling. These data are in line with previous work that has shown there is a different time-course of arterial adaptation in individuals with chronic heart failure and coronary artery disease (Maiorana et al., 2000, Walsh et al., 2003) compared to asymptomatic healthy individuals (Maiorana et al., 2001). This may be due to the impact of oxidative stress or inflammation on NO bioavailability which are known to be elevated in obese individuals with increased CVD risk (Silver et al., 2007, La Favor et al., 2016).

This is the first investigation to study the effects of exercise training on aortic PWV over 12 weeks in obese individuals with elevated CVD risk. The results mirror the changes in FMD with no change in aortic PWV following 4 weeks of training, but a significant improvement after 12 weeks. Obesity results in increased central artery stiffness even in young individuals, with subsequent negative cardiovascular outcomes (Zebekakis et al., 2005). Therefore, the improved PWV in this study may be related to improved cardiovascular risk.

Body Composition

The data demonstrate that Home-HIT is effective at improving body composition, with a reduction in whole body and visceral adiposity after 12 weeks. This was the first study to our knowledge to investigate the effect of Home-HIT on visceral fat mass following training in obese individuals. The present data are consistent with the findings of Gillen et al. (2016) who found low-volume Lab-HIT to be effective at reducing whole body and abdominal adiposity in obese/overweight women. Accumulation of whole body adipose tissue, and visceral adipose tissue in particular, is strongly associated with increased cardio-metabolic disease risk and all-cause morbidity (Poirier et al., 2006, Bogers et al., 2007), and these changes in the longer term may lead to a clinically-meaningful reduction in CVD risk and improvement in metabolic health. Interestingly, Home-HIT induced the highest mean reduction in visceral adiposity and although no statistical difference between groups was observed in the current study future work should investigate if the whole body nature of Home-HIT induces favourable reductions in visceral adiposity compared to other training methods.

Insulin Sensitivity

Home-HIT improved whole body insulin sensitivity to a similar extent as Lab-HIT and Home-MICT, and is in agreement with previous laboratory-based HIT programmes conducted in overweight/obese individuals (Cocks et al., 2016, Hood et al., 2011). The benefits of improved insulin sensitivity through Home-HIT are relevant for reducing the risk of progression to type 2 diabetes which is a major risk for macrovascular and microvascular complications that over time can lead to severe outcomes including coronary heart disease, kidney failure, limb amputations and blindness (Hellgren et al., 2015). Therefore, a high insulin-sensing capacity of skeletal muscle is required for an optimal healthy phenotype to prevent substantial perturbations in blood glucose concentrations and the long-term complications that can accompany a persistent elevation in blood glucose concentration (Abdul-Ghani et al., 2006).

Myocyte Adaptations

The myocyte adaptations investigated were selected because they are classical markers of skeletal muscle adaptation to exercise training potentially associated with improvements in health (Hawley and Lessard, 2008). Previous research has shown that HIT increases GLUT4 protein expression (Bradley et al., 2014), IMTG content (Shepherd et al., 2013a, Shepherd et al., 2017) and mitochondrial density (Shepherd et al., 2017, Tan et al., 2018). The increase in mitochondria likely underpins the improved $\dot{V}O_{2peak}$ as mitochondrial biogenesis is a major training adaptation that increases lipid and glucose fuel handling. High IMTG content is associated with insulin resistance in sedentary individuals, but athletes combine high IMTG with high insulin sensitivity (Goodpaster et al., 2001). This is explained by their greater capacity to oxidise IMTG. Therefore, the increase in mitochondria alongside increased IMTG will lead to greater IMTG oxidation during exercise and this may be linked to the improved insulin sensitivity. GLUT4 is the primary insulin-responsive

glucose transporter in skeletal muscle, and experimental increases in skeletal muscle GLUT4 expression in animal models have been shown to increase whole body insulin sensitivity (Tsao et al., 1996, Ren et al., 1995, Hansen et al., 1995). As such, the increase in GLUT4 expression likely contributed to the increased insulin sensitivity observed in the present study.

Limitations

The current pilot investigation was designed as a proof of concept study, and as such the sample size was small, providing sufficient power to detect a meaningful within group difference in $\dot{V}O_{2peak}$. Using the data generated in this study a formal power calculation suggests 69 participants per group would be required to detect a between group difference in $\dot{V}O_{2peak}$ of 1.5 $ml \cdot kg^{-1} \cdot min^{-1}$ between Home-HIT and Home-MICT. Therefore, based on this promising pilot data, future trials should investigate Home-HIT in larger cohorts to investigate its true effectiveness compared to traditional training interventions.

Conclusions

This is the first study to successfully implement an unsupervised 12-week Home-HIT training programme in previously sedentary obese individuals with elevated CVD risk. Despite being unsupervised and having no encouragement during the exercise, the Home-HIT group had high adherence rates at the prescribed exercise intensity, comparable with fully supervised Lab-HIT. Home-HIT was similarly effective at increasing a range of physiological measures to a fully supervised Lab-HIT and Home-MICT, including $\dot{V}O_{2peak}$, body mass, body fat percentage, measures of vascular function, visceral fat mass and insulin sensitivity, and markers of muscle training status all of which are indicative of a lower CVD risk. These pilot data therefore provide strong evidence that our novel Home-HIT intervention is an

effective strategy to improve cardio-metabolic health by increasing physical activity participation in the obese population most in need.

Chapter 5 A 12-Week Home-Based High-Intensity Interval Training Programme Improves Muscle Capillarisation and eNOS/NAD(P)Hoxidase Protein Ratio in Obese Individuals with Elevated Cardiovascular Disease Risk

Chapter 5

5.1. ABSTRACT

Obesity and inactivity lead to structural and functional muscle microvascular impairments associated with development of chronic disease. This study is the first to investigate the effect of a novel home-based high-intensity interval training (HIT) (Home-HT) intervention in obese individuals with elevated cardiovascular disease (CVD) risk on capillarisation and muscle microvascular eNOS/NAD(P)Hoxidase ratio. Comparisons were made with home-based moderate-intensity continuous training (Home-MICT) and supervised laboratory-based low-volume HIT (Lab-HIT) as control groups. Thirty-two sedentary obese adults (age 36±2 years; BMI 34.3±0.8 $kg \cdot m^{-2}$; $\dot{V}O_{2peak}$ 2.5±0.2 L/min^{-1}) were allocated to 12 weeks of Home-HIT (*n*=9), Home-MICT (*n*=13) or Lab-HIT (*n*=10). Muscle biopsies were taken pre- and post-training to assess specifically in the endothelial layer of muscle arterioles and capillaries the protein content of eNOS, $serine^{1177}$ phosphorylated eNOS, NOX2 and $p47^{phox}$ and various capillarisation measures using quantitative immunofluorescence microscopy. All interventions induced comparable increases in total eNOS content in terminal arterioles and capillaries (*P*<0.001). There was no change in ser^{1177} phosphorylated eNOS (arterioles *P*=0.802; capillaries *P*=0.311), but eNOS ser^{1177}/eNOS ratio significantly decreased following training in arterioles and capillaries (*P*<0.001). Training decreased NOX2 content (arterioles *P*<0.001; capillaries *P*<0.001), but there was no change in $p47^{phox}$ content (arterioles *P*=0.101; capillaries *P*=0.345). All measures of capillarisation increased (*P*<0.05). These adaptations occurred alongside increased $\dot{V}O_{2peak}$ (*P*<0.001) and whole-body insulin sensitivity (*P*=0.033). There were no significant differences between training programmes. Therefore, the training effects of Home-HIT are comparable to those of traditional training methods, with the advantage that Home-HIT reduces barriers to exercise in obese individuals with elevated CVD risk.

5.2. INTRODUCTION

With obesity and sedentary behaviour there are structural and functional impairments in the skeletal muscle microvasculature that significantly reduce the ability of the skeletal muscle to meet its metabolic demands and contribute to the development of insulin resistance and chronic diseases (Wallis et al., 2002, Clerk et al., 2006, Vincent et al., 2003). This decline in skeletal muscle microvascular function has been proposed to precede macrovascular impairments (Krentz et al., 2009). Together these observations suggest that the skeletal muscle microvasculature should be regarded as a primary target for intervention in the increasingly obese population.

Reduced skeletal muscle microvascular nitric oxide (NO) bioavailability is a central factor contributing to capillary rarefaction and the impaired vasodilatory response seen in obesity (McAllister and Laughlin, 2006, Frisbee, 2007, Olver and Laughlin, 2016). Endothelial nitric oxide synthase (eNOS) is the rate limiting enzyme responsible for NO synthesis, with the ability of eNOS to synthesise NO being determined by its protein content and activity in the endothelial layer of the muscle microvasculature (Cocks and Wagenmakers, 2016). eNOS activation is determined by phosphorylation on multiple sites, with increases in insulin, shear stress and VEGFA leading to eNOS serine^{1177} phosphorylation and vasodilation of the muscle microvasculature (Hoier et al., 2013, Hellsten et al., 2008, Mount et al., 2007, Cocks and Wagenmakers, 2016). Obesity and inactivity have been shown to alter the balance between NO production by eNOS and increase NO quenching by superoxide anions and other reactive oxygen species (ROS) (McAllister and Laughlin, 2006, Frisbee, 2005). The enzyme complex NAD(P)Hoxidase (NAD(P)Hox) has been shown to be a major source of superoxide anion production in obese individuals (Silver et al., 2007, La Favor et al., 2016). As such, the eNOS to

NAD(P)Hox protein ratio has been suggested to be a key marker of microvascular function in skeletal muscle (Cocks and Wagenmakers, 2016).

Recent work has demonstrated that 4 weeks of sprint interval training (SIT) leads to similar improvements in capillarisation and eNOS/NAD(P)Hox protein ratio as traditional moderate-intensity continuous training (MICT) in obese males (Cocks et al., 2016). Although the SIT protocol used by Cocks et al. (2016) offers a time-efficient alternative to MICT, the suitability of SIT as a safe and tolerable exercise strategy in obese individuals with elevated cardiovascular disease (CVD) risk has been questioned (Levinger et al., 2015). As such, low-volume high-intensity interval training (HIT) protocols, consisting of 60 seconds of intense constant-load cycling interspersed with 60 seconds of active recovery, have been developed as a safe and tolerable alternative (Little et al., 2011), capable of inducing similar adaptations to MICT in a number of health-related variables, including aerobic capacity ($\dot{V}O_{2max}$) and insulin sensitivity (Hood et al., 2011).

However, public health experts have questioned the applicability of current HIT interventions (Hardcastle et al., 2014, Biddle and Batterham, 2015, Courneya, 2010), arguing that although effective under optimal conditions in the laboratory (continuous supervision and specialised equipment) HIT creates a number of additional barriers to exercise in sedentary, exercise-naïve individuals in the "real world". Common barriers to current HIT protocols include difficulties with access to facilities (including travel distance and cost), expensive exercise equipment and embarrassment due to negative body image (Korkiakangas et al., 2009). To eliminate many of these barriers we recently developed a new home-based HIT (Home-HIT) intervention (**Chapter 4**) tailored to sedentary obese individuals with low fitness and mobility that could be performed in the participant's own home without supervision or equipment. In **Chapter 4** we have shown that Home-HIT was effective at overcoming many of the major barriers to exercise, offering a time-efficient, low-

cost and effective means to improve markers of cardio-metabolic health including $\dot{V}O_{2peak}$, insulin sensitivity, vascular stiffness and endothelial function. The aim of the present study was to investigate the effect of a 12-week Home-HIT intervention on skeletal muscle capillary density and skeletal muscle microvascular enzymes responsible for NO production (eNOS content and ser^{1177} phosphorylation) and NO quenching (NOX2 and p47^{phox} content) in previously sedentary obese individuals with elevated CVD risk. Comparisons were made with the effects of home-based MICT (Home-MICT) and supervised low-volume laboratory-based HIT (Lab-HIT) as control groups. We employed quantitative immunofluorescence microscopy, to assess the protein content and phosphorylation status of the indicated enzymes within the endothelial layer of the skeletal muscle microvasculature. The hypothesis was that microvascular density and eNOS content would increase to a similar extent in all three groups alongside an increase in $\dot{V}O_{2peak}$ and insulin sensitivity. The secondary hypothesis was that the three training programmes would reduce the protein content of NOX2 and its activator p47^{phox} to a similar degree in the endothelial layer of terminal arterioles and capillaries.

5.3. METHODS

The participants described in this study have been used in a previous chapter that focused on adherence and compliance to the three exercise interventions and the subsequent effect on cardio-metabolic disease risk (**Chapter 4**). Basic subject characteristics are described in **Chapter 4**, Table 4.2. The effect of the interventions on aerobic capacity and insulin sensitivity are also presented in both chapters because they are relevant for the interpretation of both studies.

Participants

Thirty-two sedentary obese adults (BMI >30 kg·m^2 or waist/hip ratio of >0.9 in men and >0.85 in women) with at least 2 further CVD risk factors, according to the American Heart Association criteria (Grundy et al., 1999), (see Table 4.1. for participant characteristics) were allocated to one of three 12-week exercise training groups: Home-HIT (n = 9); Home-MICT (n = 13); or Lab-HIT (n = 10) matched for age, BMI and $\dot{V}O_{2peak}$. Participants were free of diagnosed CVD and other contraindications to participate in an exercise intervention, ascertained through a medical screening process, and after completing the International Physical Activity Questionnaire all participants were deemed to have a sedentary lifestyle. All participants provided written informed consent, and the study was approved by the Black Country NHS Research Ethics Committee (West Midlands, UK) and conformed to the *Declaration of Helsinki*.

Pre- and Post-Training Experimental Procedures

Experimental procedures, including measures of $\dot{V}O_{2peak}$, body composition and insulin sensitivity, were undertaken before and after training (>48 h following the final exercise training session and identical in all respects to pre-training) as previously described (**Chapter 4**). Muscle samples were obtained from the vastus *lateralis* under local anaesthesia using the Bergström technique (Bergstrom, 1975) following an overnight fast. Finally, participants were provided with a physical activity monitor (ActiGraph GT3X+, Fort Walton Beach, FL) and diet diary so that habitual physical activity levels and diet could be assessed over 7 and 3 days, respectively.

Training

The 12-week training interventions started ~72 h after pre-training testing. Full details of the three training interventions (Home-HIT, Home-MICT, Lab-HIT) are described in **Chapter 4**. In brief, participants in the Home-HIT group completed repeated 1-

minute intervals composed of simple body weight exercises interspersed with 1 minute of recovery. Participants were advised to achieve ≥80% of their predicted heart rate maximum (HR_{max}; 220–age) during the intervals. During weeks 1-4 participants were advised to complete 4 intervals, this then increased by one interval per session each fortnight up to 8 intervals during weeks 11-12. In the Home-MICT group, participants performed continuous exercise of their choosing (swimming, cycling or walking/running). Participants were advised to exercise at ~65% of predicted HR_{max} throughout the sessions. During weeks 1-4 participants were asked to exercise for 30 minutes which increased by 5 minutes each fortnight up to 50 minutes during weeks 11-12. The home-based interventions were completed by the participants in an unsupervised place of their choosing outside Liverpool John Moores University (LJMU). Participants in both home-based groups were advised to train 3 times per week, but this was not enforced. To monitor the number of training sessions completed (adherence) and exercise intensity achieved (compliance), participants were given a HR monitor which was able to connect via Bluetooth to the participant's smart phone (Polar Beat; www.polar.com/beat/uk-en).

Participants in the Lab-HIT group attended the School of Sport and Exercise Sciences at LJMU to train 3 times per week under researcher supervision where they performed repeated 1-minute bouts of exercise on a cycle ergometer (Excalibur Sport V2.0, Lode, Groningen, The Netherlands), interspersed with 1 minute of active recovery. During the intervals, participants exercised at an intensity of 100% W_{max} (Little et al., 2011) in order to elicit a HR of ≥80% HR_{max}. The number of intervals were identical to the Home-HIT group.

Quantitative Immunofluorescence

NOX2 and $p47^{phox}$ protein content in the skeletal muscle microvascular endothelium and sarcolemma were assessed using the previously developed immunofluorescence staining protocol and quantification technique as described in **Chapter 3** to allow for differentiation between capillaries and terminal arterioles. Capillary and terminal arteriole specific eNOS content and eNOS ser^{1177} phosphorylation were also assessed using previously established methods (Cocks et al., 2016); however, the method was adapted to allow for assessment of individual vessel eNOS ser^{1177}/eNOS ratio to be calculated as described in **Chapter 3**.

Capillarisation

Immunofluorescence microscopy was used as described in **Chapter 3** to assess the following indexes were measured (Hepple et al., 1997): 1) the number of capillaries around a fibre (capillary contacts), 2) capillary-to-fibre ratio on an individual fibre basis and 3) capillary-fibre perimeter exchange (CFPE) index. In addition, overall capillary density was determined. Quantification of capillarisation was performed only on transverse fibres. In line with previous studies assessing capillarisation, at least 50 complete fibres were included in each analysis (Porter et al., 2002). Fibre cross-sectional area and perimeter were measured on calibrated images using ImagePro Plus 5.1 software.

Statistical Analysis

The primary aim of the study was to compare the effects of training on muscle microvascular eNOS protein content. The study was powered to detect between-group differences in these variables in response to training. G*Power 3.1 software (G*Power Software Inc., Kiel, Germany) was used to calculate the required sample size. The study was designed to detect a between-group effect of f = 0.35, representative of a medium-sized effect (Cohen, 1992), adopting an alpha of 0.05

and power of 0.80. This was deemed to be a physiologically relevant difference, as the authors have previously observed a medium effect size difference following 6 weeks of SIT and MICT in sedentary individuals (Cocks et al., 2013). As such, muscle biopsies were taken and analysed pre and post-training from 8 participants in each group. Capillary contacts, capillary-to-fibre ratio on an individual fibre basis, capillary-fibre perimeter exchange, fibre cross-sectional area and perimeter were analysed using a three-way mixed ANOVA, with the between group factor 'group' (Home-HIT vs. Home-MICT vs. Lab-HIT) and within group factors 'training status' (pre vs. post) and 'fibre type' (type 1 vs. type 2). All other variables taken pre and post-training were analysed using a 2-way mixed design ANOVA with between factor 'group' (Home-HIT vs. Home-MICT vs. Lab-HIT) and within group factor 'training status' (pre vs. post). Matsuda Index values are missing in one Lab-HIT participant and three Home-MICT participants because it was not possible to get blood samples. All analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Significance was set at $P \leq 0.05$ and data are presented as mean ± SEM.

5.4. RESULTS

General Characteristics

At baseline there were no differences in age, BMI or $\dot{V}O_{2peak}$ between groups (P=0.369; 0.455 and 0.898, respectively). Adherence and compliance (Home-HIT 96 ± 3% & 99 ± 1%; Home-MICT 88 ± 4% & 100 ± 0%; Lab-HIT 97 ± 1% & 100 ± 0%, respectively) to training did not differ between groups (see **Chapter 4** for more details). Training increased $\dot{V}O_{2peak}$ (Home-HIT 16%, Home-MICT 12%, Lab-HIT 20%) with a main effect of training (P<0.001) and no difference between groups (Table 4.2.). The Matsuda insulin sensitivity index was significantly increased after

12 weeks of training (Home-HIT 39%, Home-MICT 8%, Lab-HIT 13%; main effect of training, *P*=0.033; Table 3.1.) with no difference between groups.

Quantitative Immunofluorescence

Mean diameter of arterioles assessed were 10.2 ± 0.2 µm which is consistent with the interpretation that only terminal or 5th order arterioles were analysed (Wu et al., 2011). The mean number of arterioles analysed was 9 ± 1 at each time point per participant.

eNOS Content and Phosphorylation

Terminal arteriole eNOS content increased with training (Home-HIT = 20%; Home-MICT = 18%; Lab-HIT = 15%; main effect of training, *P*<0.001; Fig. 5.1.). There was also an increase in capillary eNOS content (Home-HIT = 21%; Home-MICT = 7%; Lab-HIT = 9%; main effect of training, *P*=0.001; Fig 5.1.). Training did not change eNOS ser^{1177} phosphorylation in the terminal arterioles (training effect, *P*=0.802) or capillaries (training effect, *P*=0.311; Fig. 3.1.). When eNOS ser^{1177} phosphorylation was normalised to eNOS content (eNOS ser^{117}/eNOS ratio) on an individual vessel basis there were significant decreases with training in the arterioles and capillaries (main effect of training, *P*=0.001 and *P*<0.001, respectively; Fig 5.1.). There were no between group differences for any of the variables.

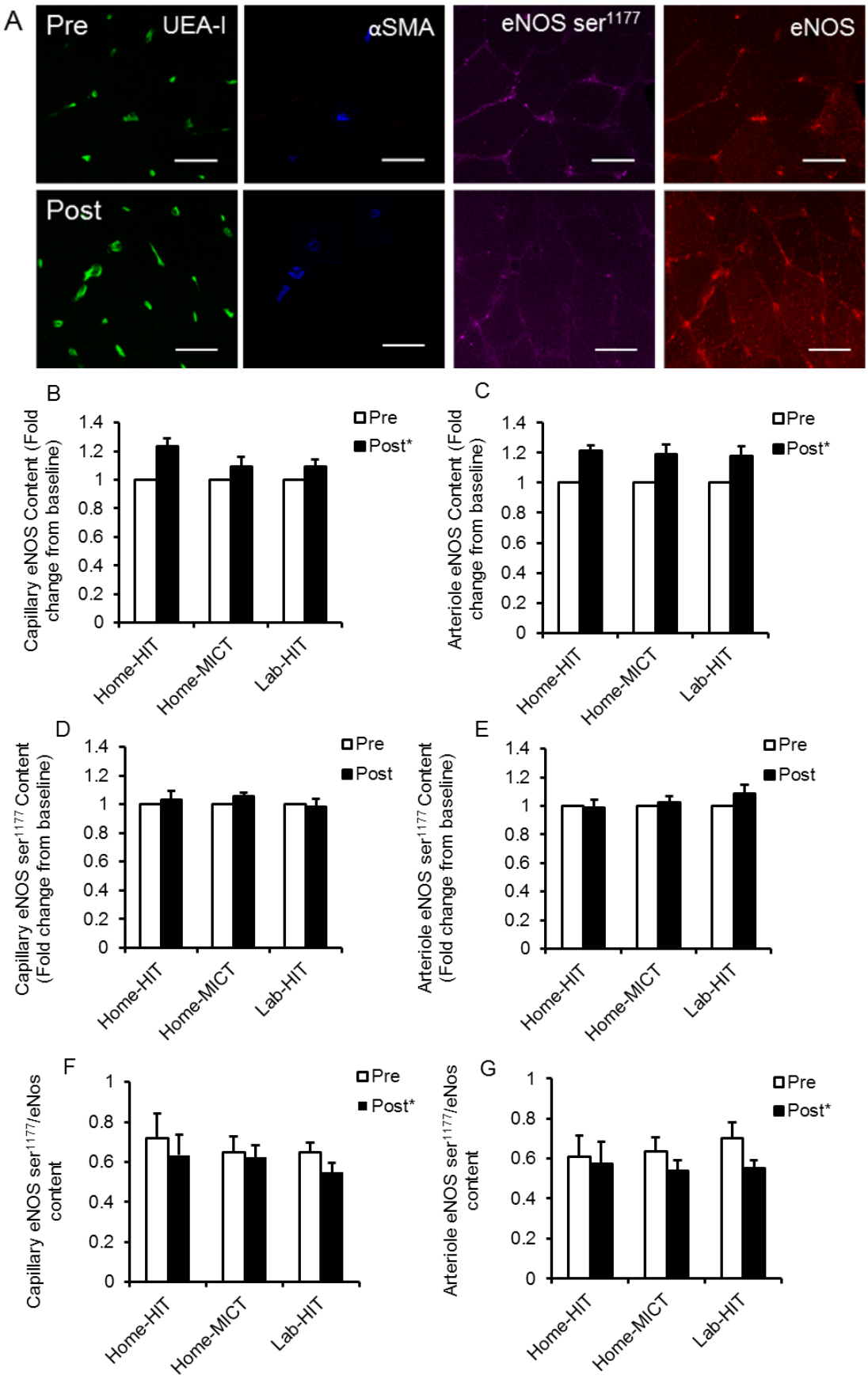
A
Pre
UEA-I
αSMA
eNOS ser1177
eNOS
Post
B
Capillary eNOS Content (Fold change from baseline)
Pre
Post*
Home-HIT
Home-MICT
Lab-HIT
C
Arteriole eNOS Content (Fold change from baseline)
D
Capillary eNOS ser1177 Content (Fold change from baseline)
E
Arteriole eNOS ser1177 Content (Fold change from baseline)
F
Capillary eNOS ser1177/eNos content
G
Arteriole eNOS ser1177/eNos content

Figure 5.1. Effect of 12 weeks of home-based high-intensity interval training (Home-HIT), home-based moderate-intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on eNOS content and eNOS ser^{1177} phosphorylation in capillaries and terminal arterioles.

(A) Representative confocal microscopy images of skeletal muscle from pre (top) and post (bottom). The skeletal muscle microvascular endothelium was revealed using *Ulex* europaeus-FITC conjugated lectin (green). Arterioles and capillaries were differentiated using anti-α-smooth muscle actin (αSMA) in combination with Alexa Fluor 405 conjugated secondary antibody (blue). Skeletal muscle eNOS ser^{1177} phosphorylation was revealed using Alexa Fluor 633 conjugated secondary antibody (purple). Skeletal muscle eNOS expression was revealed using Alexa Fluor 546 conjugated secondary antibody (red). (B) and (C) show mean fold change in eNOS content in capillaries and arterioles with training; (D) and (E) show mean fold change in eNOS ser^{1177} phosphorylation in capillaries and arterioles with training and (F) and (G) show change in eNOS/PeNOS ser^{1177} ratio with training. *Indicates a significant main effect of training (P<0.05). White bar = 50 μm.

NAD(P)Hox Subunits

Terminal arteriole NOX2 content was significantly reduced with training (Home-HIT = -22%; Home-MICT = -21%; Lab-HIT = -24%; main effect of training, P<0.001). Training also reduced skeletal muscle capillary NOX2 content (Home-HIT = -18%; Home-MICT = -14%; Lab-HIT = -24%; main effect of training, P<0.001; Fig. 3.2.). There was no change in p47^{phox} content in the terminal arterioles (P=0.101) or capillaries (P=0.345) following training. Sarcolemma-associated NOX2 and p47^{phox} content were unaltered by training (main effect of training, P=0.897 and P=0.561, respectively). There were no between group differences in any of the variables (P<0.05).

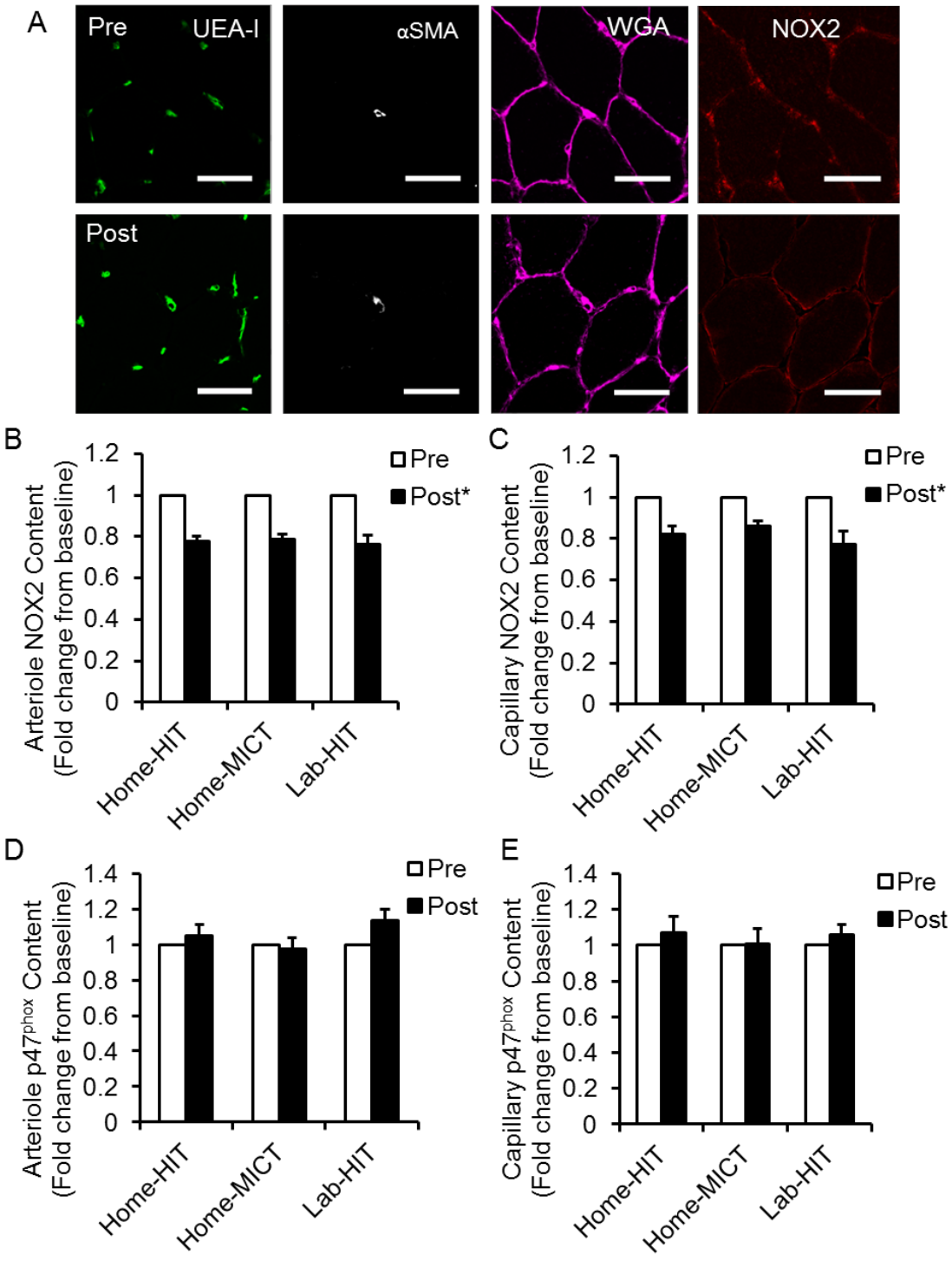

Figure 5.2. Effect of 12 weeks of home-based high-intensity interval training (Home-HIT), home-based moderate-intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on NOX2 and p47^{phox} content.

(A) Representative confocal microscopy images of skeletal muscle from pre (top) and post (bottom) training on NOX2 content. The skeletal muscle microvascular endothelium was revealed using *Ulex* europaeus-FITC conjugated lectin (green). Arterioles and capillaries were differentiated using anti-α-smooth muscle actin (αSMA) in combination with Alexa Fluor 405 conjugated secondary antibody (greyscale). Wheat germ agglutinin-633 (WGA-633; Invitrogen, Paisley, UK) was

used as a plasma marker membrane (pink). Skeletal muscle NOX2 expression was revealed using Alexa Fluor 546 conjugated secondary antibody (red). White bar = 50 µm. (B) and (C) show mean fold change in NOX2 content in arterioles and capillaries with training; (D) and (E) show mean fold change in $p47^{phox}$ expression in arterioles and capillaries with training. *Indicates a significant main effect of training (P<0.05). White bar = 50 µm.

Capillarisation

Capillary density was increased by training (Home-HIT = 15%; Home-MICT = 33%; Lab-HIT = 16%; main effect of training P<0.001), with no differences between groups (P=0.850). Capillary-to-fibre ratio, capillary-fibre perimeter exchange index and capillary contacts were all higher in type I fibres than type II fibres irrespective of training status (main effect of fibre type, P<0.05). Capillary-to-fibre ratio on an individual fibre basis (C/F_I) increased with training (Home-HIT = 16%; Home-MICT = 25%; Lab-HIT = 10%; main effect of training, P<0.001), with no difference between groups (P=0.774). Capillary-fibre perimeter exchange increased with training (Home-HIT = 14%; Home-MICT = 19%; Lab-HIT = 5%; main effect of training, P<0.001), with no differences between groups (P=0.378). Capillary contacts increased with training (Home-HIT = 15%; Home-MICT = 33%; Lab-HIT = 16%; main effect of training, P<0.001), with no difference between groups (P=0.706). There was a trend towards an effect of fibre type on fibre cross-sectional area (P=0.077), but there was no effect of fibre type on fibre perimeter (P=0.242). Training had no effect on fibre cross-sectional area (P=0.190) or perimeter (P=0.394).

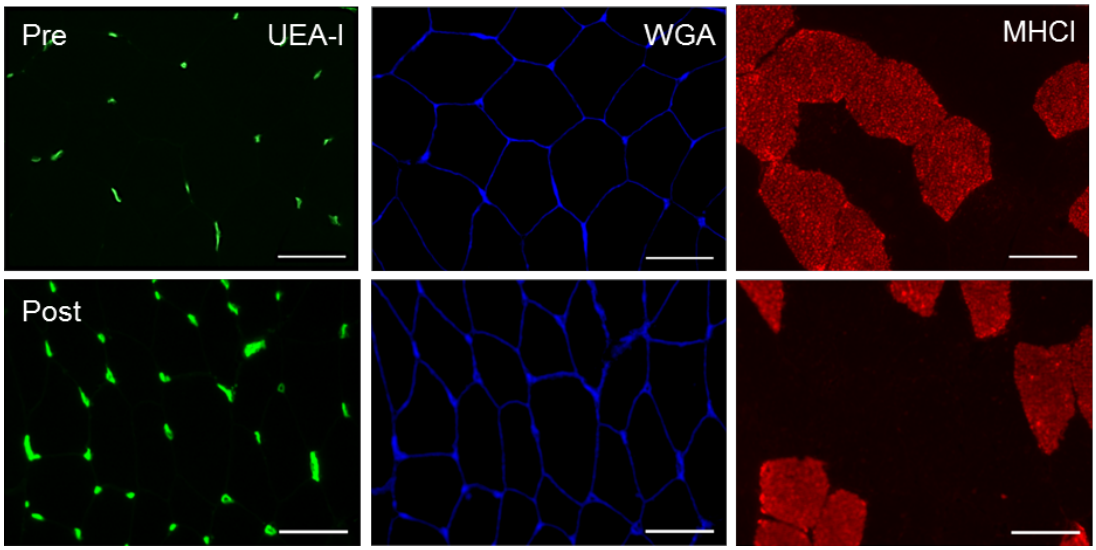

Figure 5.3. Effect of 12 weeks of home-based high intensity interval training (Home-HIT), home-based moderate intensity continuous training (Home-MICT) and lab-based HIT (Lab-HIT) on skeletal muscle capillarisation.

Representative widefield microscopy images of skeletal muscle pre (top) and post (bottom) training. Capillarisation was revealed using *Ulex* europaeus-FITC conjugated lectin (UEA-I, green). The skeletal muscle membrane was revealed using wheat germ agglutinin-633 (WGA, blue). Fibre type was revealed using anti-myosin I (MHC-I, red). White bar = 50 μm.

Table 5.1. Capillarisation pre- and post-training

	Home-HIT		Home-MICT		Lab-HIT	
Variable	Pre	Post	Pre	Post	Pre	Post
Overall FA (mm^2)	3732 ± 458	4758 ± 1231	3054 ± 342	3210 ± 342	3912 ± 716	4569 ± 1206
Type 1 FA (mm^2)	4041 ± 512	5658 ± 2065	3397 ± 286	3180 ± 266	4061 ± 761	4698 ± 1289
Type II FA (mm^2)	3550 ± 466	4277 ± 880	2852 ± 406	3192 ± 434	3901 ± 754	4509 ± 1194
Overall perimeter (mm^2)	292.2 ± 28.8	307.5 ± 33.1	253.0 ± 19.8	263.7 ± 22.5	324.7 ± 37.1	333.2 ± 41.7
Type 1 perimeter (mm^2)	300.9 ± 30.8	318.0 ± 42.7	261.4 ± 16.4	267.7 ± 26.1	329.3 ± 36.4	327.1 ± 37.5
Type II perimeter (mm^2)	287.2 ± 28.9	301.3 ± 31.5	249.1 ± 23.4	259.9 ± 23.8	323.1 ± 38.5	334.4 ± 43.4
Overall CC*	3.97 ± 0.22	4.56 ± 0.30	3.52 ± 0.42	4.68 ± 0.16	3.93 ± 0.29	4.56 ± 0.33
Type I CC*	4.33 ± 0.35	4.84 ± 0.40	3.78 ± 0.42	4.99 ± 0.20	4.35 ± 0.21	4.76 ± 0.35
Type II CC*	3.70 ± 0.20	4.27 ± 0.24	3.38 ± 0.42	4.48 ± 0.25	3.69 ± 0.36	4.44 ± 0.34
Overall *C/Fi**	1.54 ± 0.11	1.79 ± 0.12	1.41 ± 0.13	1.77 ± 0.09	1.66 ± 0.15	1.83 ± 0.17
Type I *C/Fi**	1.63 ± 0.15	1.86 ± 0.14	1.57 ± 0.13	1.89 ± 0.12	1.85 ± 0.15	1.92 ± 0.17
Type II *C/Fi**	1.47 ± 0.12	1.70 ± 0.13	1.30 ± 0.11	1.69 ± 0.12	1.54 ± 0.15	1.76 ± 0.17
Overall CFPE*	5.60 ± 0.39	6.38 ± 0.68	5.79 ± 0.53	6.90 ± 0.39	5.63 ± 0.67	5.92 ± 0.44
Type I CFPE*	5.80 ± 0.48	6.60 ± 0.79	6.20 ± 0.57	7.25 ± 0.48	6.39 ± 0.81	6.42 ± 0.64
Type II CFPE*	5.38 ± 0.33	6.10 ± 0.79	5.52 ± 0.50	6.68 ± 0.38	5.10 ± 0.55	5.65 ± 0.33
CD (caps mm^{-2})*	682.6 ± 64.9	812.7 ± 80.2	806.4 ± 78.9	955.7 ± 67.9	675.8 ± 84.2	836.6 ± 51.1

Values are mean ± SEM. *Indicates $P<0.05$, main effect of training. FA = fibre cross-sectional area, CD = capillary density, CC = capillary contacts, *C/Fi* = capillary-to-fibre ratio on an individual fibre basis, CFPE = capillary-fibre perimeter exchange.

5.5. DISCUSSION

This is the first study to demonstrate that Home-HIT, Home-MICT and low-volume Lab-HIT in obese individuals with elevated CVD risk lead to 1) increased endothelial eNOS protein content in both terminal arterioles and capillaries, 2) reduced eNOS ser^{1177} phosphorylation when normalised to the increase in eNOS content, 3) decreased endothelial NOX2 protein content in terminal arterioles and capillaries, and 4) increased skeletal muscle capillarisation. No significant differences existed between the three groups. There were also significant increases in the eNOS/NAD(P)Hox protein ratio, whole body insulin sensitivity and $\dot{V}O_{2peak}$. Importantly, Home-HIT, which has been shown to reduce the major barriers to exercise (**Chapter 4**), resulted in equal adaptations to fully supervised Lab-HIT for all these outcome measures. Therefore, Home-HIT in obese individuals with elevated CVD risk is an effective and practical training strategy capable of producing metabolic and functional adaptations in the skeletal muscle microvasculature in a direction consistent with substantial health benefits.

Skeletal Muscle Endothelial Enzymes Regulating NO Bioavailability

This is the first study to demonstrate that low-volume Lab-HIT increased terminal arteriole and capillary eNOS expression. These findings are similar to previous work from our group demonstrating that lab-based SIT increased skeletal muscle microvascular eNOS content in lean (Cocks et al., 2013) and obese (Cocks et al., 2016) individuals. However, the low-volume HIT protocol used in the current study was developed as a more suitable training method than SIT for the obese population studied due to the lower workload (Gibala et al., 2012). This study is also the first to demonstrate that the two "real world" home-based exercise programmes, performed without supervision, produced similar increases in eNOS content as the highly

controlled Lab-HIT protocol. The advantage of the Home-HIT intervention is that it simultaneously reduces many of the barriers to exercise experienced by obese exercise naïve individuals (Trost et al., 2002, Korkiakangas et al., 2009), suggesting it may be an effective alternative exercise intervention in the growing obese population. The current study found comparable increases in eNOS content in the two HIT groups and the MICT group, confirming our observations in a previous study comparing SIT and MICT in obese individuals (Cocks et al., 2016). However, an earlier study in sedentary young lean men demonstrated that the increase in eNOS was significantly greater following six weeks of "all-out" SIT than MICT (Cocks et al., 2013). This difference may have been due to variations in the training stimulus (30-second "all out" sprints vs. 1-minute submaximal exercise in the current study) and fitness differences between the studied populations.

In this chapter, eNOS ser^{1177} phosphorylation and total eNOS protein content were quantified for each microvessel in view on the same image set. This is unlike the previous publication (Cocks et al., 2012) that assessed these variables on separate muscle sections and, therefore, calculated the eNOS ser^{1177}/eNOS ratio without taking the variation in eNOS content (up to 3-fold) or phosphorylation status (up to 2-fold) between individual terminal arterioles and capillaries into account. As such, this study provides an improved quantitative assessment of these variables to training. Future studies should use this advance of the imaging method to deepen mechanistic insight in the role played by these variables and the eNOS ser^{1177}/eNOS ratio in the metabolic adaptation of the muscle microvasculature to acute stimuli (e.g. exercise, insulin and VEGF) and the training adaptations seen in trained, sedentary, obese and insulin resistant individuals.

There was no change in basal eNOS ser^{1177} phosphorylation in the microvascular endothelium following 12 weeks of training in all three groups. However, when normalised to eNOS content, eNOS ser^{1177} phosphorylation was

reduced. This is different to previous studies investigating the effect of training on basal eNOS ser^{1177} phosphorylation. Our group has previously found that 6 weeks of SIT or MICT in sedentary lean individuals reduced eNOS ser^{1177} phosphorylation expressed by itself and when normalised to eNOS content (Cocks et al., 2013). However, eNOS ser^{1177} phosphorylation was shown to increase and eNOS ser^{1177}/eNOS content ratio was unchanged in obese individuals following 4 weeks or SIT or MICT (Cocks et al., 2016). The data produced by these studies indicate that the response of eNOS ser^{1177} phosphorylation to training is affected by obesity and temporal differences. In combination, the studies suggest that in obesity eNOS ser^{1177} phosphorylation initially increases in response to training before reducing over time. This reduction in obese individuals may continue with training to eventually reduce eNOS ser^{1177} phosphorylation irrespective of eNOS content as observed in the lean individuals (Cocks et al., 2013). The decrease in eNOS ser^{1177} phosphorylation following training has been attributed to a decrease in shear stress due to the increased capillary density (Cocks et al., 2013), however Gliemann et al. (2014) suggested it may be a reflection of increased NO bioavailability as a result of less NO being scavenged by NOX2 and therefore less activation of eNOS is needed.

Expression of the catalytic subunit of the NAD(P)Hox complex NOX2 was reduced in terminal arterioles and capillaries following Home-HIT to a similar degree as Home-MICT and Lab-HIT. This adds to previous work that found 4 weeks of laboratory-based SIT and MICT reduced mixed microvascular NOX2 content in obese individuals (Cocks et al., 2016). Conversely, when investigating sedentary lean individuals, Cocks et al. (2013) found no change in mixed microvascular NOX2 protein content following SIT or MICT, presumably because the lean individuals have a very low NOX2 protein content at baseline. There was no change in arteriole or capillary content of the regulatory and assembly subunit of the NAD(P)Hox complex p47^{phox} following training. These findings agree with those of La Favor et al. (2016),

who used Western blots on whole tissue homogenates to show that 8 weeks of aerobic interval training did not alter the expression of $p47^{phox}$ in obese individuals, despite elevated baseline levels. Together the current study and that of La Favor et al. (2016) demonstrate the importance of measuring multiple NAD(P)Hox subunits to gain full insight into the effect of training on O_2^- production.

The increase in eNOS content and reduced NOX2 content following training indicates an altered balance between NO formation and quenching by O_2^- anions and other ROS leading to increased skeletal muscle microvascular NO bioavailability. Previous work has shown that NO-mediated increases in skeletal muscle perfusion are essential for optimal glucose uptake (Vincent et al., 2003, Vincent et al., 2004) and that this mechanism is impaired in obesity, contributing to impaired glucose disposal in this population (Clerk et al., 2006, Keske et al., 2009). As such, the improved eNOS/NAD(P)Hox ratio observed in the current study likely contributed to the improved insulin sensitivity observed following training. The metabolic importance of elevated eNOS content was highlighted by Kubota et al. (2011) who observed that an increase in eNOS content, through administration of bera-prost sodium (a prostaglandin I2 analogue that stimulates eNOS mRNA expression and protein synthesis), increased skeletal muscle capillary perfusion and glucose uptake in IRS-2 knockout and high-fat fed mice. In addition, obesity is associated with elevated oxidative stress in skeletal muscle due to elevated NOX-mediated ROS production, which leads to microvascular endothelial dysfunction (Weseler and Bast, 2010, La Favor et al., 2016). La Favor et al. (2016) found that 8 weeks of aerobic interval training in obese individuals decreased expression of NAD(P)Hox subunits which coincided with reduced ROS production and reversed microvascular endothelial dysfunction. The observations from these previous studies combined with the results of the present study suggest that the increased eNOS/NAD(P)Hox ratio in

obese individuals following exercise training will result in increased NO bioavailability upon insulin stimulation and a more metabolically healthy phenotype.

Capillarisation

This is the first study to demonstrate that two home-based exercise interventions performed without supervision or equipment improve capillary density, capillary contacts and capillary-fibre perimeter exchange index. The findings also extend the recent work of Tan et al. (2018), which demonstrated that 6 weeks of low-volume HIT increased capillary contacts in overweight/obese women, while we show that 12 weeks of low-volume HIT increased capillary density and capillary-fibre perimeter exchange index. The similar increases in capillarisation with Home-HIT and supervised Lab-HIT suggest Home-HIT is an effective strategy to increase capillarisation while simultaneously reducing the major barriers to exercise. The findings also provide support for previous shorter duration SIT (Cocks et al., 2013, Cocks et al., 2016) and HIT studies (Tan et al., 2018), which show no difference in fibre type specific angiogenesis in response to interval training, which is in contrast to previous work in rats showing fibre type difference in response to interval training and MICT (Gute et al., 1994).

The increase in skeletal muscle capillarisation, as shown here, is an established adaptation to exercise training that is likely to be a key contributing factor to improved $\dot{V}O_{2peak}$ (Bassett and Howley, 2000, Saltin, 1988, Andersen and Henriksson, 1977, Hellsten and Nyberg, 2015) due to prolonged mean erythrocyte transit time and decreased diffusion distance to allow increased delivery and extraction of oxygen. Increased capillarisation is also likely to contribute to the improved insulin sensitivity observed, which would improve glucose tolerance and delay progression to type 2 diabetes in obese individuals with elevated metabolic

disease risk. This is supported by Akerstrom et al. (2014), who directly investigated the effects of capillarisation on insulin sensitivity by treating sedentary rats with Prazosin (an α_1-adrenergic receptor antagonist). The ~20% increase in capillary density following 3 weeks of Prazosin treatment resulted in a ~30% increase in insulin-stimulated skeletal muscle glucose disposal, despite no change in skeletal muscle insulin signalling. This suggests that increased capillarisation with exercise training has a direct effect on insulin sensitivity, independent of other metabolic adaptations.

Conclusions

This study provides novel evidence that 12 weeks of Home-HIT, Home-MICT and Lab-HIT in obese individuals with elevated CVD risk leads to skeletal muscle microvascular adaptations that underpin the functional improvements in insulin sensitivity and $\dot{V}O_{2peak}$. All three training interventions induced similar improvements in endothelial enzyme balance as indicated by increased eNOS protein content, reduced eNOS ser^{1177}/eNOS ratio and reduced expression of the catalytic subunit of the NAD(P)Hox subunit NOX2. There was also increased capillarisation and these adaptations occurred alongside increased $\dot{V}O_{2peak}$ and whole-body insulin sensitivity. Importantly, Home-HIT induced comparable improvements in skeletal muscle capillarisation and endothelial eNOS/NAD(P)Hox balance to Lab-HIT, despite having no supervision during training. Therefore, we conclude that Home-HIT is a time-efficient and effective strategy to remove barriers to exercise and improve skeletal muscle microvascular health in the obese population most in need.

Chapter 6 A Single Bout of Fasted High-Intensity Interval Training and Moderate-Intensity Continuous Exercise Are Not Associated with a Detrimental 24-Hour Blood Glucose Profile in People with Type 1 Diabetes

Chapter 6

6.1. ABSTRACT

OBJECTIVE: To compare the effect of a bout of high-intensity interval training (HIT) with a bout of moderate-intensity continuous training (MICT) on glucose concentrations over the subsequent 24h period.

RESEARCH DESIGN AND METHODS: Fourteen people with type 1 diabetes (duration of type 1 diabetes 8.2±1.4 years), all on basal-bolus regimen, completed a randomised, counterbalanced, crossover study. Continuous glucose monitoring was used to assess glycaemic control following a single bout of HIT (6 x 1min intervals) and 30 mins of moderate-intensity continuous training (MICT) on separate days, compared to a non-exercise control day (CON). Exercise was undertaken following an overnight fast with omission of short-acting insulin. Capillary blood glucose samples were recorded pre and post-exercise to assess the acute changes in glycaemia during HIT and MICT.

RESULTS: There was no difference in the incidence of or percentage time spent in hypoglycaemia, hyperglycaemia or target glucose range over the 24h and nocturnal period (24:00-06:00h) between CON, HIT and MICT (P>0.05). Blood glucose concentrations were not significantly (P=0.49) different from pre to post-exercise with HIT (+0.39±0.42 mmol/L) or MICT (-0.39±0.66 mmol/L), with no difference between exercise modes (P=1.00).

CONCLUSIONS: HIT or 30 mins of MICT can be carried out after an overnight fast with no increased risk of hypoglycaemia or hyperglycaemia, and provided the pre-exercise glucose concentration is 7-14 mmol/L, no additional carbohydrate ingestion is necessary to undertake these exercises. As HIT is a time-efficient form of exercise, the efficacy and safety of long-term HIT should now be explored.

6.2. INTRODUCTION

Clinical guidelines recommend that people with type 1 diabetes perform at least 150 minutes of moderate-intensity physical activity per week (Colberg et al., 2016). However, a single bout of moderate-intensity exercise in people with type 1 diabetes is associated with marked decreases in blood glucose concentrations and thus an increased risk of hypoglycaemia (Ertl and Davis, 2004, Garcia-Garcia et al., 2015). The potentially large drop in blood glucose during exercise and associated fear of acute and nocturnal hypoglycaemia means that many patients avoid exercise (Lascar et al., 2014), with long-tern cardio-metabolic health consequences. Clearly, safe and effective alternative forms of exercise that minimise the perceived barriers to exercise are needed for people with type 1 diabetes.

Lack of time has also been cited as an important barrier to exercise in people with type 1 diabetes (Lascar et al., 2014). High intensity interval training (HIT), consisting of repeated bouts of high intensity exercise interspersed with low-intensity recovery, is purported as a time-efficient alternative to traditional moderate-intensity continuous training (MICT) in various groups without type 1 diabetes (Little et al., 2011). Indeed, because the typical weekly training volume during a HIT programme is approximately one third of the time commitment required for MICT (Gibala et al., 2012), HIT is able to minimise a perceived “lack of time” as a barrier to exercise. Importantly for people with type 1 diabetes, the addition of short bursts of high intensity exercise at regular intervals during a bout of MICT has been shown to assist in stabilising blood glucose concentration during exercise, and can prevent hypoglycaemia during and up to 2 hours post exercise (Guelfi et al., 2007b, Sills and Cerny, 1983). It is proposed that the increase in plasma catecholamines, growth hormone and cortisol during vigorous exercise (>80% $\dot{V}O_{2max}$) helps stabilise the glucose lowering effect of MICT (Marliss and Vranic, 2002). Therefore, in people with type 1 diabetes HIT may maintain blood glucose concentrations and reduce the risk

of hypoglycaemia both during exercise and overnight. To date, however, this has not been investigated.

The current investigation aimed to determine whether HIT maintained normoglyccaemia both during exercise and in the 24 hours following exercise. To achieve this aim, we examined the effects of a single bout of HIT and MICT following an overnight fast on acute and 24h glucose concentrations in people with type 1 diabetes, compared to a control day without exercise. Continuous glucose monitor data were analysed to assess 24h glycaemic control following exercise under controlled diet conditions using the most recent guidelines (Danne et al., 2017). Capillary blood sampling was used to assess change in blood glucose concentrations during the exercise bouts. We hypothesised that blood glucose concentrations would be maintained following HIT and that the incidence and time spent in hypoglycaemia would be lower, compared to MICT.

6.3. METHODS

Fourteen sedentary people with type 1 diabetes (6 men/8 women; age 26 ± 3 years; BMI 27.6 ± 1.3 kg·m^{-2}; $\dot{V}O_{2peak}$ 2.4 ± 0.2 L/min^{-1}; duration of type 1 diabetes 8.2 ± 1.4 years) on a basal-bolus insulin regimen completed the study. Exclusion criteria were duration of type 1 diabetes <6 months, insulin pump therapy, significant history of hyper- or hypoglycaemia (determined from medical history), obesity (BMI >30 kg·m^{-2}), pregnancy or planning pregnancy, uncontrolled hypertension (>180/100 mmHg), angina, autonomic neuropathy, taking any medication that affects heart rate, major surgery planned within 6 weeks of the study, severe nonproliferative and unstable proliferative retinopathy. Testing took place in the laboratory of the School of Sport and Exercise Sciences at Liverpool John Moores University. The study was approved by the Black Country NHS Research Ethics Committee (West Midlands,

UK) and all participants gave written informed consent to a protocol conforming to the Declaration of Helsinki.

Pre-Experimental Procedures

Participants first performed an incremental exercise test to exhaustion on an electromagnetically braked cycle ergometer (Excalibur Sport V2.0, Lode, Groningen, The Netherlands) to determine maximal aerobic power output (W_{max}) and $\dot{V}O_{2peak}$ using an online gas collection system (MOXUS modular oxygen uptake system, AEI technologies, Pittsburgh, PA). This information was used to determine appropriate workloads for subsequent exercise trials. The test consisted of 3-minute stages starting at 60 W, and the workload was increased by 35 W at each stage until subjects could not maintain a cadence of >50 rpm. $\dot{V}O_{2peak}$ was taken as the highest value achieved over a 15 second recording period. Participants also completed a food diary over a minimum of three days in order to calculate their habitual caloric and macronutrient intake.

Study Design and Experimental Protocol

Participants completed a randomised, counterbalanced, crossover experiment, consisting of 3 intervention periods: control day with no exercise (CON), HIT and MICT (see Fig. 6.1. for protocol overview). Each intervention period lasted 24h during which the effect of a single session of exercise on subsequent 24h glycaemic control and risk of hypoglycaemia were assessed under standardised dietary, but otherwise free living conditions. Periods were identical except for the exercise performed. Prior to the intervention periods participants had a Dexcom G4 Platinum CGMS (Dexcom, San Diego, CA, USA) inserted subcutaneously on the abdomen.

Participants were trained on how to use the CGMS and instructed to calibrate the device a minimum of four times daily using capillary blood tests. Participants were not blinded to the CGMS meaning they could see their glucose values. Twenty-four hours after the CGMS was inserted participants completed the control intervention. Participants did not attend the laboratory on the control day, but were provided with a standardised diet to consume while going about their normal daily activities.

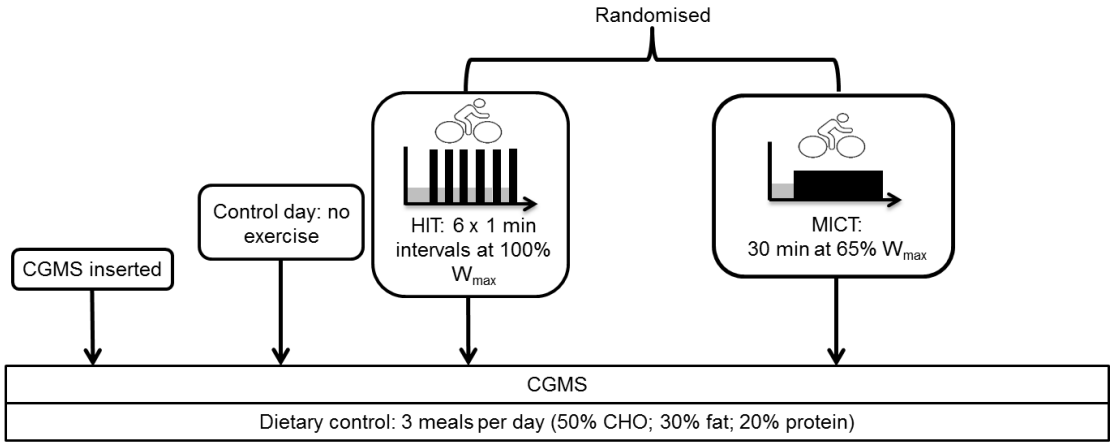

Figure 6.1. Study protocol

24h after the CGMS was inserted participants completed the control day. Participants did not attend the laboratory on the control day, but were provided with a standardised diet to consume while going about their normal daily activities. Following the control day, participants completed the two exercise intervention periods in a randomised order separated by at least 48h. The exercise intervention periods were identical to the control intervention except participants attended the laboratory following an overnight fast and having omitted their short-acting insulin to perform a bout of either high intensity interval training (HIT) or moderate intensity continuous training (MICT).

The standardised diet was matched to each participant's habitual energy intake and consisted of three meals (breakfast, lunch and dinner; 50% CHO; 30% fat; 20% protein). Participants were instructed to consume these meals at pre-determined time-points throughout the day. Participants only consumed the food provided by the

research team during this period. Additional snacks were only permitted to prevent hypoglycaemia. The diet was a 2-day rolling diet, matched for macronutrient and energy content between days, which ensured that participants consumed exactly the same food on the experimental days. Participants were also instructed to abstain from caffeine, alcohol and vigorous exercise. Participants completed a food diary to confirm that they had eaten the prescribed food at the correct times.

Following the control day participants completed the 2 exercise intervention periods in a randomised order separated by at least 48h. The exercise intervention periods were identical to the control intervention except participants attended the laboratory following an overnight fast and having omitted their short-acting insulin to perform a bout of either HIT or MICT. Following the exercise, participants left the laboratory and returned to their normal daily activities. As on the control day participants were provided with a standardised diet to consume. This diet was identical to the control day and participants consumed each meal at the same pre-determined time-points throughout the day. Insulin dosage was not recorded.

Exercise Protocols

Both exercise protocols were conducted on a stationary cycle ergometer (Excalibur Sport V2.0, Lode, Groningen, The Netherlands), and were preceded by a standardised 5 min warm-up at 50W. MICT consisted of 30 minutes continuous cycling at a workload equivalent to 65% $\dot{V}O_{2peak}$. HIT consisted of 6 x 1 minute intervals at a workload equivalent to 100% $\dot{V}O_{2peak}$, interspersed with 1 minute of rest. As such, the total time commitment of the HIT protocol (17 min) was ~half of that of the MICT protocol (35 min).

Acute Change in Blood Glucose with Exercise

Blood glucose concentration was recorded before and after exercise through capillary fingertip sampling. This was to ensure that blood glucose levels were between 7-14 mmol/L, in accordance with the guidelines we developed in the Exercising for Type 1 Diabetes (EXTOD) study (Narendran et al., 2017), meaning participants were safe to commence exercise and also safe to leave following exercise. If blood glucose was <7 mmol/L before exercise, 20g of glucose was ingested. If >14 mmol/L, a light walk or insulin was advised, as well as checking blood ketones (Riddell et al., 2017).

Statistical Analyses

Continuous glucose monitor data were downloaded from the device using Dexcom Studio™ software (12.0.4.6). Data from the CGMS were analysed in accordance to the International Consensus on Use of Continuous Glucose monitoring guidelines (Danne et al., 2017). A one-way ANOVA with repeated measures was used to assess glycaemic control between the three conditions using the following metrics: percentage of time in level 1 hypoglycaemia (≤3.9 mmol/L), level 2 hypoglycaemia (≤2.9 mmol/L), time in target range (3.9-10 mmol/L) and hyperglycaemia (≥10 mmol/L). Mean glucose and glycaemic variability using coefficient of variation were compared between conditions. Episodes of level 1 and 2 hypoglycaemia and hyperglycaemia were compared between conditions. The 24h period was defined as 08:00-08:00h and the nocturnal period was defined as 24:00-06:00h. A two factor repeated measures ANOVA was used to assess whether there was an acute change in blood glucose concentration following HIT and MICT in the fasted state with the within-subject factors ‘training mode’ and ‘time point’. All analyses were performed

using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Data are presented as mean ± SEM and significance was set at $P \leq 0.05$.

6.4. RESULTS

24h Glycaemic Control

Glucose data from the CGMS are presented in Table 6.1 and mean continuous glucose monitor traces over the 24h period in each condition are shown in Figure 6.2. The CGMS data revealed no differences in the time spent in level 1 hypoglycaemia (≤3.9 mmol/L) over the 24h period (P=0.446) or nocturnal period (P=0.944) between the CON, HIT and MICT conditions. Similarly, there were no differences in the time spent in level 2 hypoglycaemia (≤2.9 mmol/L) between the three conditions over the 24h period (P=0.518) or nocturnal period (P=0.969). There were also no differences in the time spent in target range or hyperglycaemia between the three conditions in the 24h or nocturnal periods (P>0.05).

The incidence of level 1 hypoglycaemia over the 24h period (P=0.266) and nocturnal period (P=0.522) was no different between CON, HIT and MICT. There were no differences in the incidence of level 2 hypoglycaemia over the 24h (P=0.837) or nocturnal (P=0.703) period between conditions.

There was no report of different levels of snacking between the conditions during the CGMS period. Three participants arrived to the laboratory with a blood glucose of <7 mmol/L on one trial so consumed ~200ml of Lucozade Sport Orange (20g CHO) and no participants arrived with a blood glucose >14 mmol/L.

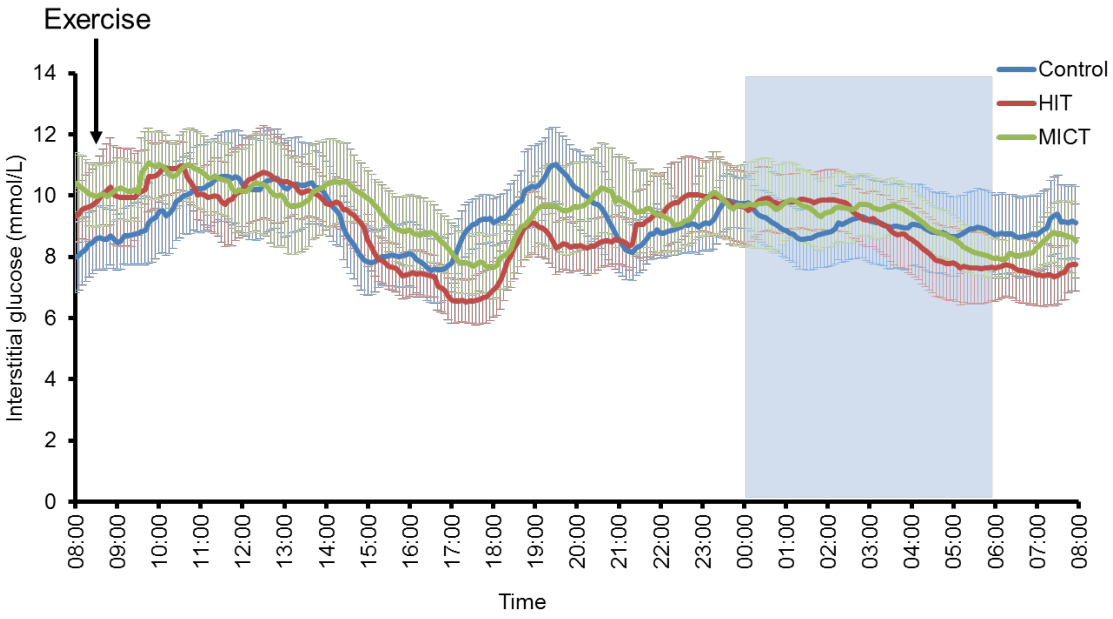

Figure 6.2. Continuous glucose monitor traces

Mean ± SEM continuous glucose monitor traces over the 24h period (08:00-08:00h) on the day of no exercise (Control), HIT and MICT. The thick lines represent the mean of all the participants' glucose traces. Exercise was performed at approximately 8:30am. The shaded grey area represents the nocturnal period (24:00-06:00h).

Table 6.1. Summary of continuous glucose monitor data

	CON	HIT	MICT
24-hr period			
Mean glucose (mmol/L)	9.2 ± 0.6	9.0 ± 0.4	9.5 ± 0.5
CV (%)	39 ± 2	39 ± 3	38 ± 3
Time in level 1 hypoglycaemia (%)	5.7 ± 1.4	7.5 ± 3.2	4.9 ± 2.0
Time in level 2 hypoglycaemia (%)	1.1 ± 0.5	3.1 ± 1.9	1.4 ± 0.7
Time in range (%)	60.5 ± 5.0	58.1 ± 3.6	59.3 ± 4.8
Time in hyperglycaemia (%)	33.7 ± 5.4	34.2 ± 3.6	35.8 ± 5.4
Incidence of level 1 hypoglycaemia	1.8 ± 0.4	2.2 ± 0.6	1.6 ± 0.5
Incidence of level 2 hypoglycaemia	0.6 ± 0.3	0.8 ± 0.4	0.6 ± 0.2
Incidence of hyperglycaemia	2.7 ± 0.3	3.1 ± 0.3	2.9 ± 0.3
Nocturnal period			
Mean glucose (mmol/L)	9.0 ± 1.0	9.0 ± 0.7	9.3 ± 0.9
CV (%)	25 ± 4	28 ± 5	19 ± 4
Time in level 1 hypoglycaemia (%)	8.9 ± 4.8	8.0 ± 3.6	7.9 ± 4.7
Time in level 2 hypoglycaemia (%)	1.5 ± 1.0	3.8 ± 2.4	4.0 ± 2.7
Time in range (%)	59.5 ± 9.7	56.8 ± 8.2	58.5 ± 8.8
Time in hyperglycaemia (%)	31.3 ± 10.2	35.1 ± 8.7	33.3 ± 9.8
Incidence of level 1 hypoglycaemia	0.4 ± 0.2	0.5 ± 0.3	0.4 ± 0.2
Incidence of level 2 hypoglycaemia	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.2
Incidence of hyperglycaemia	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1

Summary of continuous glucose monitor data for the 24h period (08:00-08:00h) and nocturnal period (24:00-06:00h) for the control day with no exercise (CON) and the days on which HIT and MICT were performed. Level 1 hypoglycaemia (≤3.9 mmol/L), level 2 (severe) hypoglycaemia (≤2.9 mmol/L), target range (4-10 mmol/L) and hyperglycaemia (≥10 mmol/L). Data are presented as mean ± SEM. There were no differences in any of the factors between the conditions (P<0.05).

Acute Change in Blood Glucose Concentration

Blood glucose concentrations did not drop during HIT (+0.39 ± 0.42 mmol/L) or MICT (-0.39 ± 0.66 mmol/L) undertaken in the fasted state (*P*=0.493), with no difference between groups (*P*=1.00; Fig. 6.3).

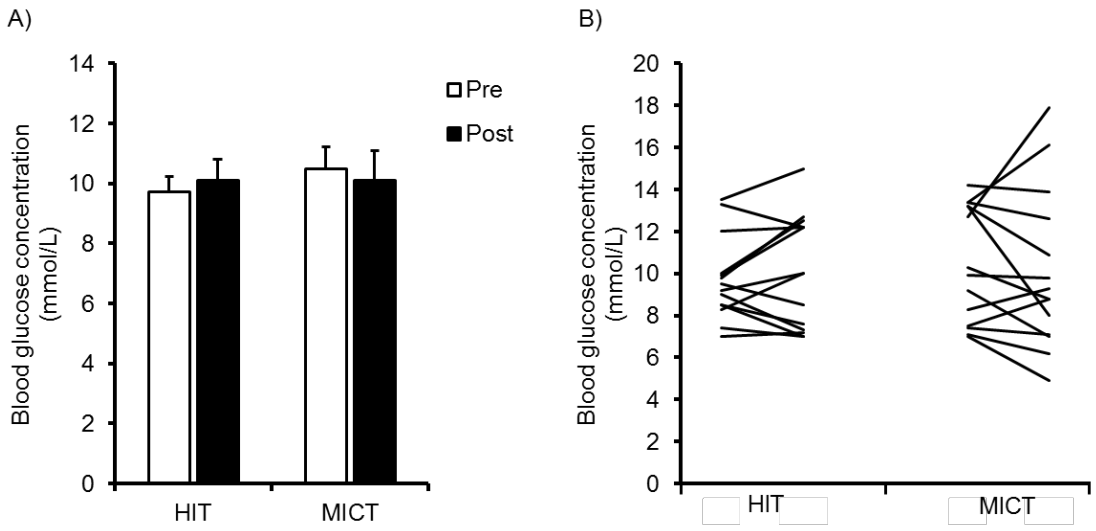

Figure 6.3. Blood glucose concentrations during HIT and MICT

Mean (±SEM) blood glucose concentrations pre and post exercise (A) and individual responses (B) to HIT and MICT sessions where the participants were overnight fasted and had omitted their fast-acting insulin.

6.5. DISCUSSION

This study examined the effects of a fasted bout of HIT and MICT on acute and 24h glucose levels in people with type 1 diabetes. The most important novel findings are that 1) there was no difference in the effect of HIT on 24h glucose compared to MICT, 2) both HIT and MICT performed following an overnight fast do not increase the incidence or time spent in hypoglycaemia over the 24h or nocturnal period in comparison to a day of no exercise, and 3) blood glucose concentration remained stable during a bout of fasted HIT and MICT. This suggests that, a single bout of HIT

or 30 minutes of MICT can be carried out after an overnight fast in people with type 1 diabetes with no increased risk of hypo- or hyperglycaemia. In addition, provided the starting glucose is between 7-14 mmol/L, our data suggest that there is no need to ingest carbohydrate during and following HIT and MICT in the fasted state.

The current exercise guidelines for people with type 1 diabetes report that aerobic exercise decreases blood glucose levels if performed during the postprandial period with insulin administration (Colberg et al., 2016). This is supported by a systematic review and meta-analysis (Garcia-Garcia et al., 2015) that aggregated the results from 10 studies to estimate rate of change in glucose concentration in response to different types of exercise in people with type 1 diabetes. Garcia-Garcia et al. (2015) reported that individuals typically experience a rapid decline in glycaemia during continuous exercise (-4.43 mmol/L h^{-1} on average), whereas the response to intermittent high intensity exercise is more varied and dependent on the protocol. In contrast, our results showed that blood glucose concentration remained stable during both HIT and MICT, and the CGMS data showed no increased risk of hypoglycaemia over the 24h period. It is likely that our results do not agree with the findings of Garcia-Garcia et al. (2015) because our study was performed in the morning following an overnight fast whereas their analysis did not control for time of day or nutritional status. Indeed, the most marked drop in blood glucose among the publications included in their meta-analysis was by Yamanouchi et al. (2002) who reported a mean drop of 4.3 mmol/L following a 30 minute walk at <50% $\dot{V}O_{2max}$ after breakfast.

The exercise guidelines published by Colberg et al. (2016) recommend that exercising while fasted may produce a lesser decrease or even a small increase in blood glucose concentration. The evidence to support this recommendation, however, is based on only one study that investigated the effects of fasted resistance training on glycaemia with no control day as a comparison (Turner et al., 2016). We

now provide the first evidence that blood glucose concentrations are stable following both HIT and MICT when undertaken after an overnight fast. The findings of the current investigation should therefore be used to inform future exercise guidelines.

The use of CGMS allowed us to compare the complete 24h glucose profiles under dietary standardisation but otherwise free-living conditions to assess whether there is a delayed response in the risk of hypoglycaemia following HIT. Fear of hypoglycaemia during and after exercise, as well as during the nocturnal period, is a major barrier to exercise for people with type 1 diabetes, so it is essential to objectively establish whether exercise increases the hypoglycaemia risk. Here we found no differences in the time spent in level 1 (≤3.9 mmol/L) or 2 (≤2.9 mmol/L) hypoglycaemia in both the nocturnal and 24h period following either HIT or MICT compared to a day of no exercise. The food diaries that participants completed indicated that they consumed the correct food and there was no difference in the amount of additional carbohydrate consumed to prevent hypoglycaemia between the conditions.

Based on our findings it appears that exercising following an overnight fast before using short-acting insulin helps to maintain blood glucose stability, irrespective of the mode or intensity of the exercise, which means that patients do not need to consume carbohydrate to avoid hypoglycaemia during exercise. However, the effects of longer duration MICT sessions will have to be tested. Future research should also investigate whether exercising regularly in the fasted state improves long-term glycaemic control. Indeed, Kennedy et al. (2013) suggested that previous research may have failed to show glycaemic benefits of exercise because calorie intake and insulin dose around the time of exercise has not been controlled. Future research which examines how exercise of different type, intensity and duration carried out in the fasted state affects 24h glucose control are needed to help to produce more flexible exercise guidelines for people with type 1 diabetes.

This is the first study to investigate the effects of HIT on glycaemia in people with type 1 diabetes. The results suggest that HIT in the fasted state offers a time-efficient exercise mode that does not increase the risk of hypoglycaemia, thus, potentially overcoming two major barriers to exercise. As typical low-volume HIT protocols require 47-60% lower time commitment to MICT sessions, this may make HIT a more attractive training strategy to potentially increase exercise uptake and adherence in people with type 1 diabetes. The efficacy of long-term HIT programmes will have to be explored in people with type 1 diabetes to determine whether this is an effective and time-efficient strategy to improve health. Furthermore, the effects of HIT in the non-fasted state have not been investigated.

The major strength of this investigation lies in the strict dietary standardisation during the CGMS period and the fact that the exercise sessions were performed at the same time of day, in the same nutritional state. Another strength is that by using CGMS we were able to study the individuals under free-living conditions, and thereby take an ecologically valid approach to investigate glucose levels following exercise. We also acknowledge that there are some limitations that will need to be addressed with further research. Firstly, we did not record insulin dose during the CGMS period and participants were not blinded to the CGMS so they may have corrected their insulin dosage or taken carbohydrate to prevent lows if they felt it was necessary. The fact that there were no differences in food intake between the days lessens the chances that change in intake could be the cause. Secondly, the small sample size makes it difficult to draw conclusions that can be applied to the wider type 1 diabetes community. However, the sample size is in line with previous investigations that have compared the glycaemic effects of different exercise intensities in people with type 1 diabetes (Turner et al., 2015, Yardley et al., 2012, Yardley et al., 2013). There were three occasions where participants arrived at the laboratory with a blood glucose of <7 mmol/L on one trial so for safety reasons they ingested ~20g CHO before the start of exercise. This highlights the difficulty of

testing people with type 1 diabetes. However, excluding these participants from the statistical analyses made no difference to the results so their data were kept in the final analysis. Finally, the MICT was only 30 minutes in duration so we do not know the effects of prolonged (>30 minutes) MICT sessions as this may lead to eventual falls in glycaemia and risk of hypoglycaemia. However, 30 minute MICT sessions are in line with the current exercise recommendations of 30 minutes on 5 days of the week (Colberg et al., 2016).

In conclusion, this is the first study to demonstrate that there is no increased risk of hypoglycaemia over the 24h period or nocturnal period following a single bout of HIT or 30 minutes of MICT in the fasted state, compared to a day of no exercise in individuals with type 1 diabetes. Secondly, blood glucose concentration is unchanged across HIT and MICT when undertaken following an overnight fast and having omitted short-acting insulin. Therefore, we recommend that in the fasted state, provided blood glucose starts between 7-14 mmol/L, carbohydrate ingestion is not needed during HIT or 30-minute MICT sessions. As HIT may offer a time-efficient and safe alternative for people with type 1 diabetes, future research should explore the efficacy of longer-term training programmes.

Chapter 7 High-Intensity Interval Training Improves Aerobic Capacity and Abolishes the Decline in Blood Glucose Observed During Moderate-Intensity Continuous Training Sessions in People with Type 1 Diabetes

Chapter 7

7.1. ABSTRACT

OBJECTIVE: To investigate whether 1) six weeks of high-intensity interval training (HIT) induces similar improvements in cardio-metabolic health markers as moderate-intensity continuous training (MICT) in people with type 1 diabetes, and 2) whether HIT abolishes acute reductions in plasma glucose observed following MICT sessions.

RESEARCH DESIGN AND METHODS: Fourteen sedentary individuals with type 1 diabetes (*n*=7 per group) completed six weeks of HIT or MICT 3 times per week. Pre- and post-training measurements were made of 24h interstitial glucose profiles (using continuous glucose monitors (CGMS)) and cardio-metabolic health markers ($\dot{V}O_{2peak}$, blood lipid profile and aortic pulse wave velocity; aPWV). Capillary blood glucose concentrations were assessed before and after exercise sessions throughout the training programme to investigate changes in blood glucose during exercise in the fed state.

RESULTS: Six weeks of HIT or MICT improved $\dot{V}O_{2peak}$ by 14% and 15%, respectively (P<0.001), and aPWV by 12% (P<0.001), with no difference between groups. 24h CGMS data revealed no differences in incidence or percentage of time spent in hypoglycaemia following training in either group (P>0.05). In the fed state, the mean change in capillary blood glucose concentration during the HIT sessions was -0.2±0.5 mmol/L, whereas blood glucose change was -5.5±0.4 mmol/L during MICT.

CONCLUSIONS: Six weeks of HIT improved $\dot{V}O_{2peak}$ and aortic PWV to a similar extent as MICT. The finding that blood glucose remains stable during HIT in the fed state, but consistently falls during MICT, suggests that HIT may be the preferred training mode for people with type 1 diabetes.

7.2. INTRODUCTION

Regular exercise is recommended for people with type 1 diabetes to maintain overall health and reduce the risk of macrovascular and microvascular complications, which are a major cause of mortality and morbidity (Chimen et al., 2012, Moy et al., 1993). The current guidelines for people with type 1 diabetes are to undertake at least 150 minutes of moderate to vigorous aerobic exercise per week, spread over at least three days per week, with no more than two consecutive days without activity (Colberg et al., 2016). Benefits of exercise for those with type 1 diabetes include improved aerobic capacity ($\dot{V}O_{2max}$), insulin sensitivity, body composition, endothelial function and blood lipid profile (Chimen et al., 2012, Makura et al., 2013, Codella et al., 2017, Pang and Narendran, 2008). Despite the benefits, few people with type 1 diabetes achieve exercise targets and many programmes designed to increase physical activity have failed (Brazeau et al., 2014, Bohn et al., 2015). In addition to the barriers to exercise cited by the general population, such as a perceived lack of time, work commitments and cost (Trost et al., 2002), people with type 1 diabetes face additional barriers including fear of hypoglycaemia, loss of glycaemic control and inadequate knowledge around exercise management (Lascar et al., 2014, Brazeau et al., 2008).

To overcome a perceived lack of time, high-intensity interval training (HIT) is purported as a time-efficient alternative to moderate-intensity exercise to improve numerous cardio-metabolic risk factors including $\dot{V}O_{2max}$, insulin sensitivity and glycaemic control in people without type 1 diabetes (Hood et al., 2011, Little et al., 2011). Furthermore, results from **Chapter 6** show that a single bout of HIT does not increase the risk of hypoglycaemia in people with type 1 diabetes. Whether HIT offers a safe, effective and time-efficient training strategy to improve cardio-metabolic health that reduces the risk of hypoglycaemia in people with type 1 diabetes is yet to be investigated.

Here we investigated the hypothesis that six weeks of HIT would improve markers of cardio-metabolic health, including $\dot{V}O_{2peak}$, glycaemic control, blood lipid profile and vascular health in people with type 1 diabetes. A moderate-intensity continuous training (MICT) group was used as a control. During this 6-week training period capillary blood glucose concentrations were monitored before and after exercise sessions to provide further information on the acute effects of HIT and MICT on blood glucose concentration.

7.3. METHODS

Fourteen previously sedentary people with type 1 diabetes (10 men/4 women; see Table 5.1. for subject characteristics) on a basal-bolus insulin regimen completed six weeks of supervised HIT (*n*=7) or MICT (*n*=7) three times per week. Participants were pair-matched based on sex, age and $\dot{V}O_{2peak}$ to the two training groups. Exclusion criteria were duration of type 1 diabetes <6 months, insulin pump therapy, significant history of hyper- or hypoglycaemia (determined from medical history), obesity (BMI >30 $kg \cdot m^{-2}$), pregnancy or planning pregnancy, uncontrolled hypertension (>180/100 mmHg), angina, autonomic neuropathy, taking any medication that affects heart rate, major surgery planned within 6 weeks of the study, severe nonproliferative and unstable proliferative retinopathy. Testing took place in the laboratory of the School of Sport and Exercise Sciences at Liverpool John Moores University. The study was approved by the Black Country NHS Research Ethics Committee (West Midlands, UK) and all participants gave written informed consent to a protocol conforming to the *Declaration of Helsinki*.

Pre-Training Assessments

Participants first performed an incremental exercise test to exhaustion on an electromagnetically braked cycle ergometer (Excalibur Sport V2.0, Lode, Groningen, The Netherlands) to determine maximal aerobic power output (W_{max}) and $\dot{V}O_{2peak}$ using an online gas collection system (MOXUS modular oxygen uptake system, AEI technologies, Pittsburgh, PA) as described in **Chapter 3.** Participants also completed a food diary over a minimum of three days in order to calculate habitual caloric and macronutrient intake.

Three to 7 days after the incremental exercise test, participants attended the laboratory after an overnight fast (>10 h) for a second pre-training assessment session. Following 15 minutes rest, supine brachial artery blood pressure measurements were made in triplicate using an automated sphygmomanometer (GE DINAMAP Pro 300 V2). Aortic pulse wave velocity (aPWV) measurements were made using a semi-automated device and software (SphygmoCor, AtCor Medical, Sydney, Australia), as previously described by Cocks et al. (2016) and **Chapter 3**. A fasting blood sample was used to determine fasting plasma cholesterol and triglyceride concentrations, using a semi-automatic spectrophotometer (Randox RX Daytona™, County Antrim, UK).

A Dexcom G4 Platinum (Dexcom, San Diego, CA, USA) CGMS was inserted subcutaneously on the abdomen. A habitual free-living 24h glucose profile was analysed at least 24 hours after the CGMS was inserted. Participants were trained to use the CGMS and instructed to calibrate the device a minimum of four times daily using capillary blood tests. Participants were provided with a standardised diet of three meals (breakfast, lunch and dinner) during the CGMS period (50% CHO; 30% fat; 20% protein) in accordance with their habitual calorie intake. Participants were instructed to consume these meals at pre-determined time points throughout the day.

No additional snacks were permitted and participants only consumed the food provided by the research team during this period, unless they needed to prevent hypoglycaemia (blood glucose <3.0 mmol/L) (International Hypoglycaemia Study Group, 2017). A food diary was completed to confirm that they had consumed the prescribed food at the correct times. Participants were instructed to avoid alcohol and caffeine, as well as exercise throughout the CGMS period.

Exercise Training

Training started ~72h after completion of the pre-experimental procedures. Participants trained three times per week for six weeks under researcher supervision on a Lode Corival cycle ergometer (Corival *Lode BV, Groningen, The Netherlands)*. Following a 3-minute low-intensity warm-up, the HIT group performed repeated 1 minute bouts of high intensity cycling at a workload equivalent to 100% $\dot{V}O_{2peak}$ interspersed with 1 minute of recovery at 50 W, whereas the MICT group performed continuous moderate intensity cycling at a workload equivalent to 65% $\dot{V}O_{2peak}$. The number of intervals in the HIT group increased from 6 in weeks 1 and 2, to 8 in weeks 3 and 4 to 10 in weeks 5 and 6. The duration of the sessions in the MICT group were 30 minutes in weeks 1 and 2, 40 minutes in weeks 3 and 4 and 50 minutes in weeks 5 and 6.

Acute Change in Blood Glucose with Exercise

Before starting and after completing each training session during the six-week training period, participant's blood glucose concentrations were required to be between 7-14 mmol/L, in accordance with the Exercising for Type 1 Diabetes (EXTOD) guidelines (Narendran et al., 2017). If blood glucose concentrations fell outside of this range corrective measures were taken; glucose was ingested if blood

glucose <7 mmol/L, and a light walk or insulin bolus was advised if glucose >14 mmol/L, as well as checking blood ketones (Riddell et al., 2017). During the MICT sessions participants were advised to check their blood glucose concentrations part-way through the exercise and to consume carbohydrate as necessary to prevent hypoglycaemia. Capillary blood glucose concentrations were recorded immediately before and after exercise. As such, over the course of the six weeks of training we gathered pre and post-exercise blood glucose concentrations from a total of 108 MICT training sessions and 87 HIT sessions in the fed state. The proportion of total sessions that blood glucose was recorded was 86% in the MICT group and 69% in the HIT group.

Post-Training Assessments

Approximately 72h after the final training session, participants attended the laboratory on two occasions (separated by 72h) to complete a series of post-training assessments. These assessments were identical in all respects to those undertaken prior to training (pre-training assessments).

Statistical Analyses

The primary outcome variable to measure a significant training benefit was $\dot{V}O_{2peak}$. Previous research in our group (Shepherd et al., 2015, Shepherd et al., 2013a) has suggested a SD of 2.7-3.2 to detect a change in $\dot{V}O_{2peak}$ of 3.5 $ml \cdot kg^{-1} \cdot min^{-1}$, which is a clinically significant increase in $\dot{V}O_{2peak}$ (Myers, 2003). A power calculation suggested that 7-9 participants were required in each group to detect a within-group difference with a paired *t* test with 80% power at a significance level of 0.05. Continuous glucose monitor data were downloaded from the device using Dexcom Studio™ software (12.0.4.6) and analysed in accordance with the International

Consensus on Use of Continuous Glucose Monitoring (Danne et al., 2017). Glycaemic thresholds were defined as follows: target range (3.9-10 mmol/L), level 1 hypoglycaemia (≤3.9 mmol/L), level 2 hypoglycaemia (≤2.9 mmol/L) and hyperglycaemia (≥10 mmol/L). The 24h period was defined as 08:00-08:00h and the nocturnal period was defined as 24:00-06:00h. All variables were analysed using a two-way mixed ANOVA, with the between factor 'group' (HIT vs. MICT) and repeated factor 'training status' (pre-training vs. post training), followed by Bonferroni *post-hoc* corrections. A two way mixed ANOVA, with the between factor 'group' and the repeated factor 'time point' (pre-training vs. post training) was used to assess whether there was an acute change in blood glucose concentration following HIT and MICT in the fed state over the 6 weeks of training. The CGMS did not work on one participant in the MICT group. Aortic PWV readings were obtained from five participants in the HIT group and six in the MICT group. All analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Data are presented as mean ± SEM and significance was set at $P \leq 0.05$.

7.4. RESULTS

By design, there were no differences in age ($P=0.877$), $\dot{V}O_{2peak}$ ($P=0.371$) or duration of type 1 diabetes ($P=0.291$) between the training groups at baseline. BMI was, however, significantly higher in the HIT group compared to the MICT group ($P=0.038$). Pre and post-training variables are presented in Table 5.1. Training increased $\dot{V}O_{2peak}$ (HIT 14%, MICT 15%; $P<0.001$) and W_{max} (HIT 13%, MICT 14%; $P<0.001$), with no difference between groups (Fig. 7.1). Six weeks of training also improved aPWV ($P=0.001$) and there was no difference between groups. Systolic, diastolic and mean arterial blood pressure did not improve following training ($P=0.219$; $P=0.476$; $P=0.268$, respectively). There was no change in plasma

cholesterol or triglyceride concentrations with training (*P*=0.881; *P*=0.652, respectively).

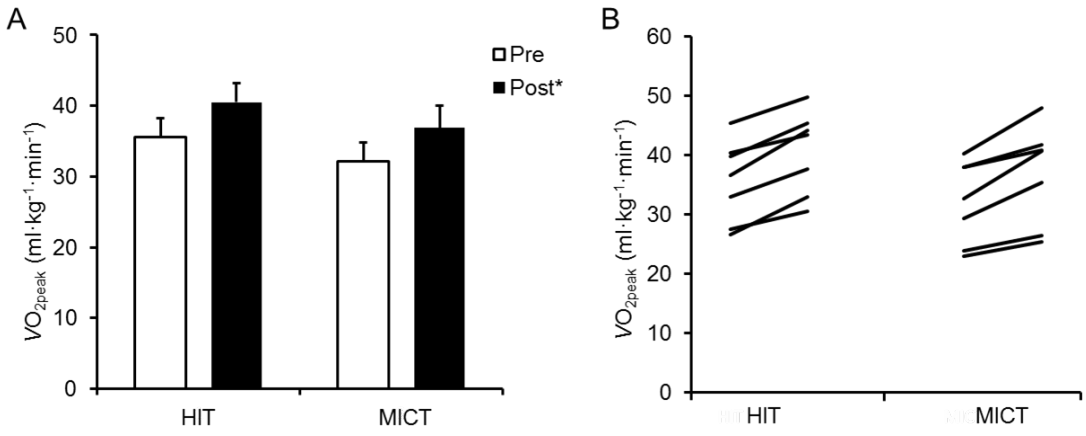

Figure 7.1. Effect of six weeks of high intensity interval training (HIT) and moderate intensity continuous training (MICT) on $\dot{V}O_{2peak}$.

(A) Shows the mean responses and (B) shows individual responses in $\dot{V}O_{2peak}$ with training. *Indicates a significant difference from baseline (*P*<0.05).

Table 7.1. General characteristics

	HIT		MICT	
	Pre	Post	Pre	Post
Age (years)	29 ± 3	-	29 ± 5	-
Sex	5M/2F	-	5M/2F	-
Duration of T1D (years)	13 ± 3	-	9 ± 2	-
Mass (kg)	90.0 ± 4.8	89.8 ± 4.8	76.7 ± 5.4	76.3 ± 5.3
BMI ($kg \cdot m^{-2}$)	29.2 ± 1.2	29.2 ± 1.2	25.3 ± 1.2	25.2 ± 1.2
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	35.6 ± 2.6	40.5 ± 2.6*	32.1 ± 2.6	36.9 ± 3.2*
$\dot{V}O_{2peak}$ (L/min^{-1})	3.2 ± 0.3	3.7 ± 0.3*	2.5 ± 0.3	2.9 ± 0.4*
Wmax (W)	245 ± 16	277 ± 19*	202 ± 22	231 ± 24*
SBP (mmHg)	121 ± 3	119 ± 4	123 ± 4	122 ± 4
DBP (mmHg)	65 ± 3	63 ± 3	70 ± 5	68 ± 4
MAP (mmHg)	84 ± 3	82 ± 2	87 ± 4	86 ± 3
aPWV (m/s)	6.1 ± 0.5	5.4 ± 0.7*	6.1 ± 0.4	5.4 ± 0.4*
Cholesterol (mmol/L)	5.07 ± 0.29	5.12 ± 0.35	4.81 ± 0.41	4.93 ± 0.41
Triglycerides (mmol/L)	0.94 ± 0.09	1.03 ± 0.25	0.70 ± 0.04	0.65 ± 0.06

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; aPWV = arterial pulse wave velocity. Data are presented as mean ± SEM. *Denotes a significant change from pre-training to post-training ($P<0.05$).

Glycaemic Control

Glucose data from the CGMS obtained over a 24h period pre and post training are presented in Table 7.2. There was no difference in the time spent in level 1 hypoglycaemia (≤3.9 mmol/L) over the 24h period (P=0.727) or nocturnal period (P=289) with training. Similarly, there was no difference in time spent in level 2 hypoglycaemia (≤2.9 mmol/L) with training over the 24h period (*P*=0.442) or nocturnal period (*P*=0.397). There were also no differences in the time spent in target range over the 24h (*P*=0.412) or nocturnal periods (*P*>0.382). Furthermore, there was no difference in the time spent in hyperglycaemia over the 24h (*P*=0.540) or nocturnal period (*P*=0.118). However, there was an interaction effect for the time spent in target range (*P*=0.034) and time in hyperglycaemia over the nocturnal period (*P*=0.039). Post hoc analysis revealed that the HIT group spent significantly less time in target glycaemia during the nocturnal period (*P*=0.038) which was due to a greater time spent in hyperglycaemia over the nocturnal period (*P*=0.016). The incidence of level 1 hypoglycaemia over the 24h period (*P*=0.675) and nocturnal period (*P*=0.363) was no different with training. There were no differences in the incidence of level 2 hypoglycaemia over the 24h (*P*=0.174) or nocturnal (*P*=0.549) with training.

Table 7.2. Summary of continuous glucose monitor data

	HIT		MICT	
	Pre	Post	Pre	Post
24h period				
Mean glucose (mmol/L)	9.3±0.3	9.5±1.0	9.2±0.6	8.6±0.7
CV (%)	42.6±3.6	38.2±2.6	37.9±3.6	36.9±4.0
Time in level 1 hypoglycaemia (%)	6.1±2.6	5.4±3.1	3.4±1.5	2.8±1.9
Time in level 2 hypoglycaemia (%)	0.2±0.2	0.5±0.3	0.9±0.5	0.0±0.0
Time in range (%)	56.7±3.1	56.4±7.8	59.3±5.8	68.2±7.7
Time in hyperglycaemia (%)	37.0±2.0	37.7±8.9	36.3±6.5	28.9±8.5
Incidence of level 1 hypoglycaemia	1.8±0.6	1.2±0.5	0.9±0.5	1.4±0.6
Incidence of level 2 hypoglycaemia	0.2±0.2	0.2±0.2	0.4±0.2	0.1±0.1
Incidence of hyperglycaemia	3.0±0.5	2.8±0.5	3.2±0.5	2.5±0.5
Nocturnal period				
Mean glucose (mmol/L)	8.8±1.3	11.7±2.0	8.0±1.2	7.4±1.1
CV (%)	23.2±5.9	19.5±9.1	29.1±7.2	22.2±6.2
Time in level 1 hypoglycaemia (%)	9.3±9.0	3.0±2.0	7.6±5.0	4.9±4.9
Time in level 2 hypoglycaemia (%)	0.0±0.0	1.2±1.2	3.2±2.0	0.0±0.0
Time in range (%)	57.4±15.5	32.6±14.3*	60.0±15.4	71.3±16.5
Time in	33.3±16.7	63.0±16.3*	28.5±15.9	23.6±15.8

hyperglycaemia (%)				
Incidence of level 1 hypoglycaemia	0.5±0.2	0.3±0.2	0.3±0.2	0.1±0.1
Incidence of level 2 hypoglycaemia	0.0±0.0	0.2±0.2	0.3±0.2	0.0±0.0
Incidence of hyperglycaemia	0.5±0.2	0.8±0.2	0.5±0.2	0.3±0.2

The 24h period was defined as 08:00-08:00h and nocturnal period as 24:00-06:00h. Level 1 hypoglycaemia (≤3.9 mmol/L), level 2 (severe) hypoglycaemia (≤2.9 mmol/L), target range (3.9-10 mmol/L) and hyperglycaemia (≥10 mmol/L). There were no differences in any of the variables with training (P>0.05).

Acute Change in Blood Glucose during Training Sessions

When quantifying the change in blood glucose concentration during exercise training sessions undertaken in the fed state over the six-week intervention, the mean change in blood glucose concentration in response to HIT was -0.2 ± 0.5 mmol/L (P<0.001) whereas blood glucose decreased by -5.5 ± 0.4 mmol/L in response to MICT (P=0.626; Fig. 7.2).

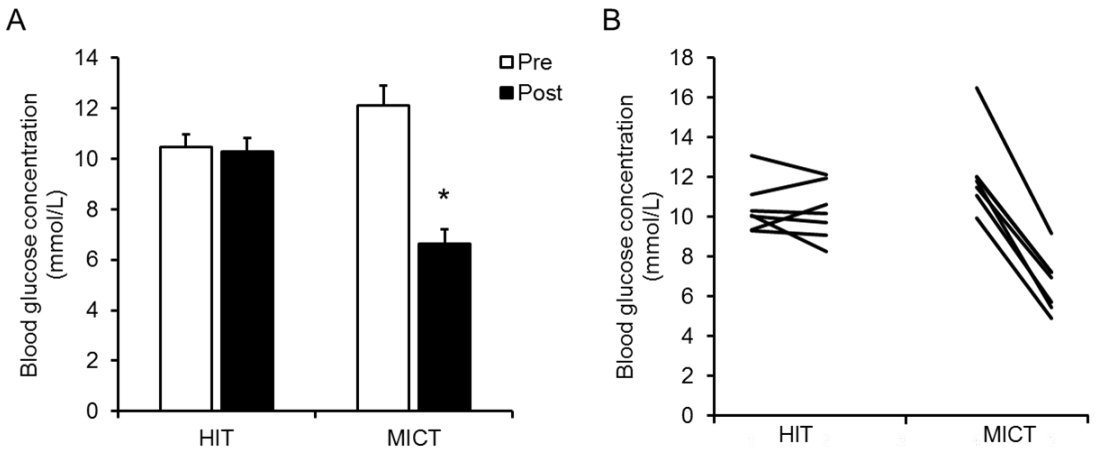

Figure 7.2. Change in blood glucose following exercise in the fed state

Finger prick blood glucose concentrations were recorded immediately before and after exercise. As such, over the course of the six weeks of training we gathered pre and post exercise blood glucose concentrations from a total of 108 MICT training sessions and 87 HIT sessions in the fed state (86% and 69% of total possible sessions, respectively). Mean change in blood glucose concentration (A) and average change in blood glucose concentration during HIT and MICT over the 6 week training period (B). *Denotes a significant change from baseline (P<0.05).

7.5. DISCUSSION

This study demonstrates for the first time that six weeks of HIT improves $\dot{V}O_{2peak}$ and aPWV in people with type 1 diabetes to a similar magnitude as MICT. Secondly, we observed that blood glucose concentration remained stable during the HIT sessions performed in the fed state throughout the training programme, but there was a consistently large drop in blood glucose during MICT throughout the training programme, with participants at risk of hypoglycaemia. The CGMS data revealed that there was a decrease in the time spent in target range in the HIT group which was due to an increase in time spent in hyperglycaemia while there were no changes in glycaemic control in the MICT group. The fact that HIT improved $\dot{V}O_{2peak}$ and aPWV to a similar extent as MICT but did not cause a fall in glucose during exercise

suggest that HIT may be a more practical exercise strategy for patients with type 1 diabetes.

Aerobic capacity improved to a similar extent following six weeks of HIT and MICT, despite the weekly time commitment being 54-90 minutes less for HIT than for MICT. The 14% increase in $\dot{V}O_{2peak}$ observed in our investigation following HIT (a mean increase of 4.9 ml·kg^{-1}·min^{-1}) is high in comparison to other studies using similar protocols that tend to report changes of 7-10% in populations without type 1 diabetes (Weston et al., 2014) and the only other study to examine the effect of sprint interval training in type 1 diabetics (repeated 30-second maximal cycling bouts interspersed with 3-4 minutes of rest 3 times a week for 7 weeks) reported a 7% increase in $\dot{V}O_{2peak}$ (Harmer et al., 2008). This has clinical importance given that $\dot{V}O_{2max}$ is reported to be the strongest prognostic marker of cardiovascular mortality (Myers, 2003) and improvements in $\dot{V}O_{2max}$ with exercise training are associated with a reduction in all-cause mortality risk (Lee et al., 2010). In fact, Myers (2003) found that there is a 8-17% reduction in all-cause mortality for each 1-MET (~3.5 ml·kg^{-1}·min^{-1}) increase in $\dot{V}O_{2max}$. Although these correlations have not been specifically confirmed in people with type 1 diabetes, it is likely that the HIT programme used here induces clinically meaningful benefits to this population, which is especially important as they are at increased risk of cardiovascular disease compared to a non-diabetic population (Chimen et al., 2012, Moy et al., 1993).

In the present study there was a 12% reduction in aPWV following both training modes, which is greater than has previously been reported in other training studies in populations without type 1 diabetes (Slivovskaja et al., 2017, Horner et al., 2015). To the authors' knowledge, this is the first study to investigate changes in arterial stiffness following HIT and MICT in people with type 1 diabetes. The reduction in aPWV is of clinical relevance as increased arterial stiffness is associated with negative cardiovascular outcomes (Cecelja and Chowienczyk, 2009).

Neither training mode improved glycaemic control according to the CGMS data, measured as time spent in target range (euglycaemia) or hypoglycaemia or the incidences of hypoglycaemia. Previous studies using HbA1c and daily insulin dosage as a marker of glycaemic control have also failed to show overall improvements in glycaemic control with exercise training (Laaksonen et al., 2000, Lehmann et al., 1997, Harmer et al., 2008), although studies reporting positive effects of training on glycaemic control do exist (Salem et al., 2010). There was a reduction in the time spent in euglycaemia in the HIT group which was due to an increase in the time spent in hyperglycaemia. Although increasing the proportion of time spent in hyperglycaemia during the nocturnal period is not desirable, it did reduce the risk of developing hypoglycaemia. This effect of HIT needs to be explored further as an increase in time spent in hyperglycaemia could increase the risk of long-term complications. It could be speculated that participants in the HIT group consumed additional carbohydrates before going to bed to prevent hypoglycaemia and as a result spent more time in hyperglycaemia. However, the study was performed under strict dietary control and participants in the HIT group did not report consuming additional snacks. The small sample size and the fact that just one 24h period was recorded pre and post-training in each individual is more likely to account for these differences. Although the use of CGMS in our investigation allowed a detailed analysis of glycaemic control, we acknowledge that longer duration exercise training programmes with larger sample sizes are needed to assess the effects of exercise training on long-term glycaemic control. Furthermore, the current guidelines suggest that a minimum of 14 consecutive days should be recorded when analysing CGMS data (Danne et al., 2017). Unfortunately, these guidelines were published after our data collection was completed so will be used in future studies.

Before the training sessions, we recorded blood glucose concentration for safety reasons to prevent participants from exercising when glucose concentrations were too high or low based on the EXTOD guidelines (Narendran et al., 2017). Blood

glucose was also recorded after the sessions so that participants did not leave the laboratory while they were potentially at increased risk of hypoglycaemia. This meant that we collected pre and post-exercise blood glucose readings from up to 18 training sessions for each participant over the course of six weeks of HIT or MICT. We found that blood glucose concentration remained stable during HIT throughout the training programme, but there was a consistently large drop in blood glucose during MICT, with participants at risk of hypoglycaemia. This was a consistent observation across all participants undertaking MICT (Fig. 7.2b). The changes in blood glucose concentration during the exercise we have reported here are striking and are the first of their kind in the literature over so many training sessions. Furthermore, they are supported by Garcia-Garcia et al. (2015) who conducted a systematic review and meta-analysis in which they aggregated results from 10 studies to estimate rate of change of glucose concentration during and after different types of exercise in people with type 1 diabetes. Their results showed a rapid decline in glycaemia during continuous exercise (-4.43 mmol/L h^{-1} on average) while the results were more variable during intermittent high intensity exercise depending on the protocol.

The drop in blood glucose concentration during the MICT sessions is likely due to the effects of short-acting insulin in the circulation. In healthy individuals, blood glucose concentration remains stable during moderate-intensity aerobic exercise because insulin secretion is suppressed progressively with exercise duration and there is a gradual increase in glucagon and adrenaline resulting in increased hepatic glucose production (Marliss and Vranic, 2002, Wasserman et al., 1991). Therefore, contraction-mediated glucose uptake is matched by increased hepatic glucose production so that blood glucose concentration remains stable at ~4.0-6.0 mmol/L (Marliss and Vranic, 2002). However, as insulin is supplied exogenously in people with type 1 diabetes, hyperinsulinaemia is likely to occur

because of increased blood flow and mobilisation of insulin from its subcutaneous depot, particularly if the injection site is in an exercised region (Marliss and Vranic, 2002). This results in enhanced glucose uptake due to combined contraction-mediated and insulin-stimulated GLUT4 translocation. The high insulin levels will also suppress the exercise-mediated increases in glucagon and adrenaline and their ability to stimulate hepatic glucose production (Guelfi et al., 2007a). As a result, muscle glucose uptake during MICT will exceed hepatic glucose production, leading to the large decreases in plasma glucose concentration observed in this study (Fig. 7.2.). Hyperinsulinaemia has also been shown to suppress adipose tissue and intramuscular triglyceride (IMTG) lipolysis in healthy individuals (Coyle et al., 1997), which will reduce the contribution of lipids to the fuel mixture oxidised during exercise. The combination of insulin and exercise-mediated glucose disposal coupled with decreased hepatic glucose production and reduced lipolysis and lipid oxidation increases the risk of hypoglycaemia during MICT. On the other hand, the stable blood glucose concentrations during HIT are likely due to increased plasma catecholamines which offset the effects of hyperinsulinaemia through increased hepatic glucose output. Previous research has shown that addition of a sprint to a bout of moderate-intensity exercise in individuals with type 1 diabetes opposed the fall in glycaemia during exercise and this was associated with a rise in catecholamines (Bussau et al., 2006). Following a bout of HIT, it may be speculated that the greater catecholamine response compared to MICT may lead to stimulation of adipose tissue lipolysis and increase oxidation of the released fatty acids in the muscle during recovery (Watt et al., 2003).

Another important observation, although not quantitatively reported here, was the number of training sessions in which participants had to prevent or treat an episode of hypoglycaemia by consuming fast-acting carbohydrate. During the MICT sessions, participants were advised to stop exercising at least once to check their blood glucose concentration in accordance with the EXTOD guidelines (Narendran

et al., 2017), correct accordingly with glucose if necessary, and then wait for their blood glucose to stabilise before recommencing the training. Many of the participants in the MICT condition found this frustrating and it would often mean that the already time consuming 50-minute cycling sessions were even longer while blood glucose was checked. The large drop in blood glucose concentration that we found during the MICT sessions highlights why the guidelines recommend that carbohydrate should be taken when doing more than 30 minutes of moderate-intensity exercise (Riddell et al., 2017).

The main strengths of this investigation were 1) the strict dietary standardisation under free-living conditions during the CGMS period pre and post-training, and 2) the monitoring of acute changes in blood glucose concentrations during exercise throughout the intervention. We also acknowledge that there are some limitations. The sample size of the study was small; however, the clear significant increases in $\dot{V}O_{2peak}$ suggest that we have the power to conclude that HIT is effective at improving $\dot{V}O_{2peak}$ in people with type 1 diabetes. Secondly, we did not record insulin dose before and after the training intervention. This would be useful to determine whether there is a change in insulin sensitivity as reduced insulin dosage is associated with decreased risk of cardiovascular complications in people with type 1 diabetes (Schauer et al., 2011, Bergman et al., 2012a).

In summary, this is the first study to demonstrate that six weeks of HIT leads to comparable improvements in $\dot{V}O_{2peak}$ and arterial stiffness to MICT. HIT though may be the preferred exercise approach, as blood glucose remains stable during HIT, but falls substantially during MICT. We therefore recommend that HIT in the fed state is a safe, effective, flexible and time-efficient form of exercise for people with type 1 diabetes.

Chapter 8 A Multi-Disciplinary Evaluation of Home-Based High-Intensity Interval Training in People with Type 1 Diabetes: A Pilot Study

Chapter 8

8.1. ABSTRACT

OBJECTIVE: To use a multi-disciplinary approach to evaluate a home-based high-intensity interval training (Home-HIT) intervention in people with type 1 diabetes.

RESEARCH DESIGN AND METHODS: Eleven individuals with type 1 diabetes (4 men/7 women; age 30±3 years; BMI 27.1±1.2 $kg \cdot m^{-2}$; $\dot{V}O_{2peak}$ 2.5±0.2 L/min^{-1}; duration of type 1 diabetes 10 ± 2 years) completed six weeks of Home-HIT. The effect of Home-HIT on $\dot{V}O_{2peak}$, blood pressure, insulin dose and glycaemic profile was assessed pre and post-training. Adherence and ability to meet target heart rate (HR) thresholds (compliance) were monitored using a HR monitor and mobile phone application. Change in glycaemia was measured pre, post and 1h post exercise sessions throughout the six-week period. A qualitative online survey was completed post-training.

RESULTS: Training adherence was 95±2% and compliance was 99±1%. Six weeks of Home-HIT increased $\dot{V}O_{2peak}$ by 8% (P=0.018), decreased insulin dose by 13% (P=0.026), but did not affect blood pressure (P=1.000). There was no change blood glucose from baseline immediately or 1h post Home-HIT sessions throughout the intervention. Qualitative responses about the programme were generally positive, providing support that Home-HIT removes barriers to exercise.

CONCLUSIONS: This is the first study to combine physiological outcomes with a qualitative evaluation of a training intervention in people with type 1 diabetes. Home-HIT resulted in high adherence alongside increased $\dot{V}O_{2peak}$ and decreased insulin dose. Because Home-HIT is time-efficient and removes barriers to exercise including fear of hypoglycaemia, it may represent an effective strategy to increase exercise participation in people with type 1 diabetes.

8.2. INTRODUCTION

People with type 1 diabetes are recommended to engage in regular exercise due to its recognised physiological and psychological benefits (Chimen et al., 2012, Moy et al., 1993, Wasserman and Zinman, 1994). The current guidelines for people with type 1 diabetes are to accumulate 150 minutes of moderate to vigorous aerobic exercise per week, spread over at least 3 days, with no more than 2 consecutive days without activity (Colberg et al., 2016). However, many people with type 1 diabetes lead a sedentary lifestyle and fail to meet these guidelines (Makura et al., 2013, Tielemans et al., 2013, Waden et al., 2008), with lack of time and fear of hypoglycaemia identified as key barriers to exercise (Lascar et al., 2014, Brazeau et al., 2008).

Recent work from our laboratory has demonstrated that unlike traditional moderate-intensity continuous training (MICT), high-intensity interval training (HIT) does not cause acute reductions in blood glucose concentrations during exercise in people with type 1 diabetes (**Chapter 7**). Furthermore, 6 weeks of HIT in people with type 1 diabetes led to similar increases in $\dot{V}O_{2peak}$ and aortic pulse wave velocity to MICT, despite training volume being 47-60% less (**Chapter 7**). However, during this study, HIT was performed under optimal conditions with high levels of researcher supervision and specialised equipment, meaning the “real world” effectiveness of HIT in people with type 1 diabetes is unclear. The HIT protocol did not address a number of other key barriers to exercise such as difficulties with access to facilities (including distance and cost) and embarrassment due to negative body image (Lascar et al., 2014). To combat these traditional exercise barriers, another study included in this thesis demonstrated the effectiveness of a novel home-based HIT (Home-HIT) programme in previously sedentary obese individuals with elevated cardiovascular disease risk (**Chapter 4**). This Home-HIT protocol, which could be performed without equipment in a place of the participant’s choosing, resulted in high

adherence, suggesting it successfully removed many of the aforementioned barriers to exercise. It was also effective at improving a number of cardio-metabolic health markers such as $\dot{V}O_{2peak}$, insulin sensitivity, endothelial function and body composition. For people with type 1 diabetes, Home-HIT may offer an exercise strategy that reduces the two major barriers to exercise, lack of time and fear of hypoglycaemia, but is also inexpensive and requires no facilities, equipment or travel time to gyms.

To date very little evidence has looked to qualitatively explore participant experiences of HIT (Kinnafick et al., 2018), and no studies have investigated the attitudes and barriers of HIT in people with type 1 diabetes in conjunction with an exercise programme designed to increase exercise participation. Therefore, the inclusion of a qualitative survey would allow a more in-depth exploration of participant experiences of the Home-HIT intervention to support the assessment of physiological effectiveness.

The overall aim of this pilot study was to investigate the potential of Home-HIT to be a practical and effective training method for people with type 1 diabetes. To achieve this aim, participants completed a 6-week Home-HIT intervention investigating 1) adherence and compliance (defined as ability to meet prescribed heart rates), 2) the effect of Home-HIT on $\dot{V}O_{2peak}$, 3) the effect of Home-HIT on insulin dose and glycaemic profile during the first and last week of training, and 4) the acute effect of Home-HIT on blood glucose concentration following each training session. Finally, participants completed an online survey to gain greater understanding of the barriers to exercise that people with type 1 diabetes face as well as participant experiences of the Home-HIT intervention.

8.3. METHODS

Subjects

Eleven previously physically inactive individuals with type 1 diabetes (6 on insulin pumps/5 on multiple daily injections) completed the intervention (see Table 8.1 for characteristics). Exclusion criteria were duration of type 1 diabetes <6 months, significant history of hyper- or hypoglycaemia, pregnancy or planning pregnancy, uncontrolled hypertension (>180/100 mmHg), angina, autonomic neuropathy, taking any medication that affects heart rate (HR), major surgery planned within 6 weeks of the study, severe nonproliferative and unstable proliferative retinopathy. The study was approved by the Black Country NHS Research Ethics Committee (West Midlands, UK) and all participants gave written informed consent to a protocol conforming to the *Declaration of Helsinki*.

Table 8.1. Pre-training individual participant characteristics

Characteristic	Values	Range
Age (years)	30 ± 3	20 - 55
Sex (M/F)	4/7	-
BMI ($kg \cdot m^{-2}$)	27.1 ± 1.2	21.5 – 32.9
Duration of type 1 diabetes (years)	10 ± 2	1 – 16
Treatment (pump/MDI)	6/5	-
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	32.4 ± 2.1	23.7 – 45.1
$\dot{V}O_{2peak}$ (L/min^{-1})	2.5 ± 0.2	1.7 – 4.2
Systolic blood pressure (mmHg)	126 ± 4	106 – 142
Diastolic blood pressure (mmHg)	78 ± 4	62 – 95
Mean arterial blood pressure (mmHg)	94 ± 3	78 - 111

MDI = multiple daily injections. Data presented as mean ± SEM.

Pre-Training Testing

Participants attended the laboratory at Liverpool John Moores University (LJMU) for one pre-training visit. Following 15 minutes supine rest blood pressure was recorded in triplicate using an automated sphygmomanometer (Dianamap; GE Pro 300V2, Tampa, Florida). Participants then completed an incremental exercise test to exhaustion on an electromagnetically braked cycle ergometer (Excalibur Sport V2.0, Lode, Groningen, the Netherlands) to determine $\dot{V}O_{2peak}$ using an online gas collection system (MOXUS modular oxygen uptake system, AEI technologies, Pittsburgh, PA). The test consisted of 3-minute stages starting at 60 W, with workload increasing by 35 W at each stage until subjects could not maintain a cadence of >50 rpm. $\dot{V}O_{2peak}$ was taken as the highest value obtained over a 15 second recording period.

Training

The six-week home-based training programme started ~72h after completion of pre-training testing. Training was completed in an unsupervised place of the participant's choosing, outside of the LJMU laboratories and is fully described in **Chapter 4**. In brief, participants were asked to perform repeated 1 minute bouts of high intensity exercise interspersed with 1 minute of rest. Participants were advised to achieve a HR of ≥80% of their predicted maximum HR (220 – age) at some point throughout the intervals. The 1 minute intervals were composed of two 30-second simple bodyweight exercises with no rest in between. Participants were provided with 18 exercises with 9 suggested exercise pairs which were detailed in an exercise pack. Participants were free to choose from all of these pairs during the sessions.

To monitor training session completion rate (adherence) and exercise intensity achieved (compliance), participants were given a HR monitor which was

able to connect via Bluetooth to the participant's smart phone (Polar Beat; www.polar.com/beat/uk-en). Following each training session HR data was automatically uploaded to a cloud storage site (www.flow.polar.com), which was monitored by the researchers to check adherence and compliance. Participants were aware that the research team were monitoring their training, but this software was not used to enforce adherence or compliance. Instead, participants were advised to complete 3 sessions per week and it was their responsibility to schedule and complete the sessions. The number of intervals increased from 6 in weeks 1 and 2, to 8 in weeks 3 and 4, to 10 in weeks 5 and 6. To avoid participant drop-out participants were contacted by text/email once every two weeks to enquire about progress and any general issues around training. If participants were observed to have missed consecutive training sessions their email enquired as to whether there was a specific reason, however no direct encouragement was given for them to re-engage with training.

Acute Effect of Exercise on Blood Glucose Control

Participants were asked to record their blood glucose pre, post and 1h post each training session so that change in blood glucose could be monitored over the 18 sessions. Participants were also asked to record whether they required additional carbohydrates or insulin during or following the session to adjust their blood glucose. Throughout the programme participants were asked to ensure that blood glucose levels were between 7-14 mmol/L, in accordance with the guidelines developed for the Exercising for Type 1 Diabetes (EXTOD) study (Narendran et al., 2017), meaning that they were safe to commence exercise. If blood glucose was outside of this range participants were asked to postpone the exercise.

Effect of Training on Glycaemic Profile and Insulin Dose

During the first and final 7 days of the training programme participants were asked to monitor their blood glucose and insulin dose using an 8-point profile: before and 2h after each meal, just before bed, and at 2am. This was designed to provide information on whether the training programme had an effect on daily insulin dose and glucose control over the week.

Post-Training Testing

Approximately 72h after the final training session, participants attended the laboratory for a post-training visit. Here $\dot{V}O_{2peak}$ and blood pressure were re-assessed. Participants also completed a post-training qualitative survey.

Qualitative Survey

During post-training testing, participants completed an online qualitative survey (www.surveymonkey.co.uk) anonymously while alone in a quiet space. The survey included 7 open and 3 closed questions that asked participants' about their barriers and facilitators to exercise, their experiences of the Home-HIT training programme and their intentions to exercise in the future (Table 8.2). Questions were developed, piloted within and revised by the research team using appropriate literature (Kinnafick et al., 2018). Participants were given the following instructions: "Please read the following questions and write your answers in the space provided. Give as much or as little information as you like".

Table 8.2. Qualitative survey questions

1. Prior to taking part in the Home-HIT study, how would you describe your activity levels?
2. How did you feel about the level of activity you engaged in prior to the programme?
3. Have you ever played team sports or exercised with others (for example at classes)?
4. Are there any aspects of exercise (type 1 related or not) that you feel might prevent you from taking part?
5. The current health recommendation is that we all undertake a minimum of 150 minutes of exercise weekly as 30 minute sessions, five times a week. How do you feel about this? What may help or hinder you?
6. Would any of the following encourage you to exercise? • Being given free access to a gym • Being given a programme that could be completed within your home • Attending an exercise group organised by the hospital or your GP • One to one advice outlining an exercise programme • Being provided with a mobile phone application detailing an exercise programme, with video examples • A leaflet outlining an exercise programme • One to one advice on each session and how to progress the programme • A mobile phone application that gives feedback on each session and how to progress the programme • Self-monitoring an exercise programme having being given advice in a leaflet
7. How do you feel about HIT style exercise after completing this study?
8. Please describe what you did and did not like about the programme.
9. Are there any benefits or drawbacks to HIT at home?
10. Would you consider continuing HIT at home? Or would you prefer to try something else?

Qualitative Analysis

The answers from the qualitative survey were analysed using a framework approach (Ritchie and Spencer, 1994) as this is a flexible approach appropriate for multi-disciplinary research teams (Gale et al., 2013). The analytical process was guided by Gale and colleagues' (Gale et al., 2013) 7 stages of analysis (given that responses were already transcribed, the process started at stage 2). The stages included; familiarisation with the interview, coding of the responses, developing an analytical framework to group and discuss codes (three of the researchers discussed this on two occasions); applying the analytical framework where agreed groups of codes were applied to the transcript; charting data to the framework where quotes were aligned to an appropriate group of codes; and finally interpreting the data where researchers discussed the meaning of quotes, consulted notes collected by F.K. during the analytical process and agreed on how they would be used to support the physiological data.

Statistical Analysis

Changes in $\dot{V}O_{2peak}$, blood pressure, glycaemic profile and insulin dose were assessed with paired samples *t* tests. Change in blood glucose concentration pre, post and 1h post exercise was assessed with a one-way ANOVA. Analysis was done using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Significance was set at $P \leq 0.05$ and data are presented as mean ± SEM.

8.4. RESULTS

Adherence and Compliance to Home-HIT

Mean training session adherence was 95 ± 2% (range = 83-100%) with participants achieving the 80% HR_{max} target in 99 ± 1% of sessions (range = 94-100%).

Aerobic Capacity and Blood Pressure

$\dot{V}O_{2peak}$ increased by 8% (P=0.018) following 6 weeks of Home-HIT (Figure 8.1). There was no change in systolic (126 ± 4 vs. 125 ± 4 mmHg; P=0.975), diastolic (78 ± 4 vs. 78 ± 4 mmHg; P=0.967) or mean arterial (94 ± 3 vs. 94 ± 3 mmHg; P=1.000) blood pressure following training.

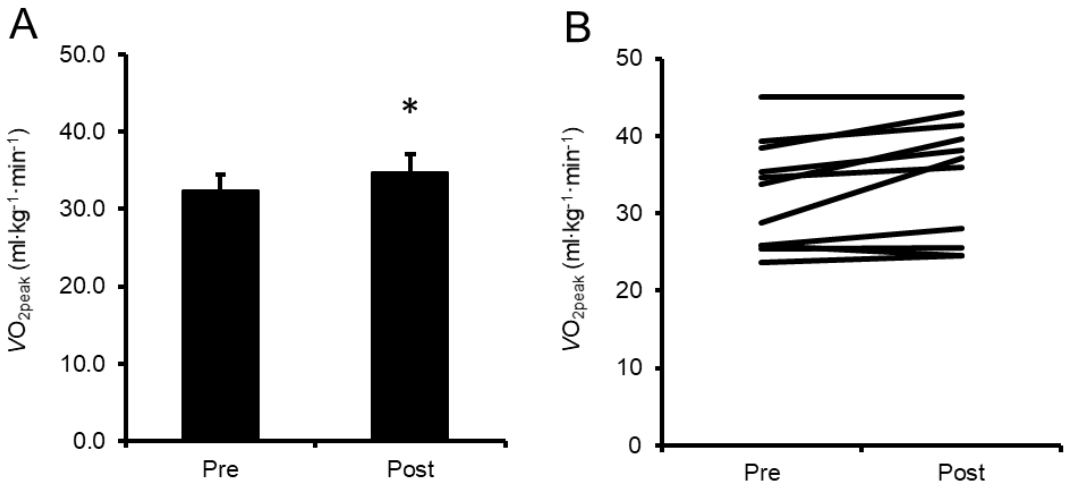

Figure 8.1. Change in $\dot{V}O_{2peak}$ following 6 weeks of home-based high intensity interval training (Home-HIT)

A) The group mean response in $\dot{V}O_{2peak}$ following training. *Indicates a significant change from baseline (P<0.05). B) Shows the individual changes in $\dot{V}O_{2peak}$ with training.

Blood Glucose Control and Insulin Dose

Blood glucose results pre, post and 1h post exercise are shown in Figure 8.2. Mean blood glucose concentration immediately post exercise and 1h post exercise were not different from baseline (*P*=0.070). Participants needed to correct their blood glucose concentrations with consumption of carbohydrates to prevent hypoglycaemia in 6 ± 3% of sessions (10 out of 188 sessions) and for hyperglycaemia with insulin in 2 ± 1% of sessions (3 out of 188 sessions). Everyone was able to complete every session without stopping mid-way to check their blood glucose concentration.

The 8-point insulin diary showed that mean short acting insulin dose significantly decreased by 13% from 0.31 ± 0.06 to 0.27 ± 0.05 IU/kg/day post training (*P*=0.026). There was no change in mean blood glucose concentration per day (pre = 8.8 ± 0.5 mmol/L; post = 8.6 ± 0.4 mmol/L; *P*=0.457) and there was no difference in glucose variation, measured as coefficient of variation, pre and post training (pre = 34 ± 3%; post = 32 ± 3%; *P*=0.307).

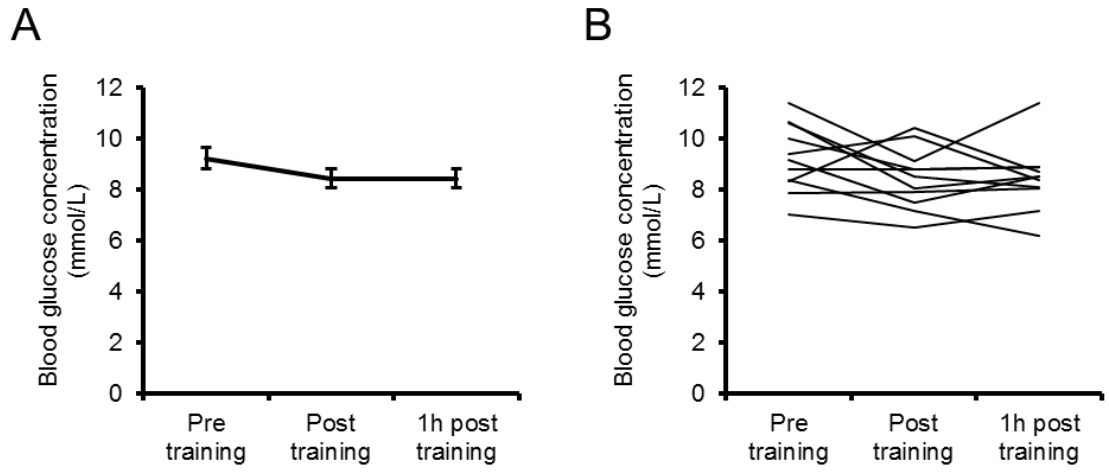

Figure 8.2. Change in blood glucose concentration during training sessions

A) Shows the group mean blood glucose concentrations over the course of the training sessions. B) Shows mean blood glucose concentration for each participant over the 6 weeks of Home-HIT pre, post and 1h post training. There was no significant difference in blood glucose concentration across time points (*P*>0.05).

Qualitative Survey

Three key themes, and sub-themes, were developed during analysis. These themes included; 1) *Flexibility of Home-HIT* with the sub-themes *Type 1 Related Flexibility* and *Non-Type 1 Diabetes Related Flexibility*, 2) *Motivation*, and 3) *the Exercise Experience*.

Theme 1: Flexibility of Home-HIT

Participants all agreed that Home-HIT provided them with flexibility not offered by other forms of exercise. Comments around flexibility centred on issues regarding their diagnosis and issues that were separate to their diagnosis.

Type 1 Diabetes Related Flexibility:

Participants disclosed that previously they had experienced difficulty in managing blood glucose during exercise, especially if it was over a long duration or in a competitive environment.

> *"From experience exercise that requires stamina causes me to have a hypo during or after the activity."* (Participant 5)

Three participants stated that Home-HIT appeared to reduce the number and severity of their hypos. These participants reported that the control over their blood glucose levels reduced the worry associated with a hypoglycaemic incident.

"I feel that HIT style exercises work very well for me in terms of improving my overall fitness and reducing the occurrence of hypos whilst exercising." (Participant 8)

"The even blood glucose levels are an absolute dream come true for exercise with T1. I'd even try it of an evening and go to bed less worried." (Participant 6)

However, one participant said that Home-HIT made their hypos more frequent and severe.

"Demanding physically, but particularly made blood sugar control more difficult and hypos more frequent and severe." (Participant 9)

Non-Type 1 Diabetes Related Flexibility:

Common positives of the Home-HIT programme included the low time commitment, ease of doing the exercises at home, having a good variety of exercise, being able to monitor HR on their mobile phone and not having to go to the gym, all of which made Home-HIT more flexible. The small time commitment required during Home-HIT was mentioned in over half of the participants' responses, with many saying it was easy to fit within a busy lifestyle because it could be done at home with no equipment. Just one person said they found it difficult to find the time to fit the training in because of their busy work schedule. Lack of time was identified as a major barrier to exercise before the programme with demanding work hours and family commitments being a major problem.

"Time constraints are a massive barrier to achieving this (government's physical activity guidelines) *for me. I'd have to cut time off from other things and it would impact on my work/life balance."* (Participant 6)

> *"It was very easy to fit the workout sessions into my day, depending on what I was doing due (to) the time it took to complete."* (Participant 8)

One person also mentioned that at times they found it difficult to exercise at home because they would get distracted easily. Participants who expressed poor perceptions of body image, from weight gain, or low competence for exercise were grateful for the flexibility of being in the home where no one could see them and they did not feel judged.

> *"Being able to complete the programme in my own flat, without feeling self-conscious about people watching what I was doing."* (Participant 8)

Theme 2: Motivation

Participants' responses to the survey described their motivation prior, during and for future engagement. A low level of motivation, and sometimes complete lack of motivation, were cited as reasons for not taking part in regular exercise prior to taking part in Home-HIT. In regards to Home-HIT, three people said that they particularly liked having the target number of intervals to complete each session. Four individuals responded that self-monitoring via the instant feedback of the HR monitor and mobile app and having a variety of exercises provided encouragement to continue.

> *"I would consider doing HIT at home if I could view my progress through a monitor device like a HR monitor. It did not take long to complete each session and I feel as though my blood sugar control is better doing HIT x3 a week."* (Participant 5)

Wanting a source of social support was mentioned by several participants. One person said that they would have liked someone with them while doing the exercises to time the intervals to keep them motivated. Two participants explained that they liked being able to choose which exercises they completed and how the number of intervals increased as they progressed through the programme.

> *"I liked the opportunity to choose which exercises to do during each session and that I was able to monitor my own progress through a heart rate monitor device through my phone. I also liked how throughout the programme the intensity increased and this became a challenge."* (Participant 5)

Two participants explained that it would have been hard to motivate themselves to complete the programme unless they felt they were being monitored by the research team using the mobile app.

Theme 3: The Exercise Experience

When asked to provide information on barriers and facilitators to the programme, most participants provided details of their experiences of the exercise itself. Participants agreed that they liked the interval style exercises as they found they did not get bored. Three participants stated that they found the training programme very demanding, especially when it increased to 10 intervals. However, they acknowledged that it was rewarding when they had finished.

> *"I liked the interval training as you do not get a chance to become bored if you have a set training program to follow."* (Participant 4)

> *"It was initially hard to complete but got better as the sessions continued and it was good to measure my heart rate and see if any progress was being made."* (Participant 2)

Two of the individuals said they would have liked a greater variety of exercises, whereas others felt the variety was sufficient. When asked if they would consider continuing the Home-HIT programme after the study 10 out of the 11 said that they would. Eight of these said that they would continue the programme alongside other forms of exercise.

> *"I would continue the HIT at home but would be interested in trying something new."* (Participant 8)

8.5. DISCUSSION

This is the first study to combine physiological data with qualitative evaluation of a training intervention in people with type 1 diabetes. We provide strong evidence that Home-HIT is an effective intervention with high adherence and compliance, leading to increases in $\dot{V}O_{2peak}$ despite participants being unsupervised during the exercise. Home-HIT appears to reduce traditional barriers to exercise as well as fear of hypoglycaemia, which is the major barrier in people with type 1 diabetes. Therefore, Home-HIT may represent an effective strategy to increase exercise participation in people with type 1 diabetes.

Six weeks of Home-HIT increased $\dot{V}O_{2peak}$ by 8% (0.2 L/min^{-1}). This is similar to previous laboratory-based HIT studies in healthy individuals (Weston et al., 2014); however, not as high as the 14% increase that we reported in our laboratory-based HIT study in people with type 1 diabetes (**Chapter 7**). Aerobic capacity has been shown to be a more powerful predictor of mortality than clinical risk factors such as

hypertension, smoking and type 2 diabetes (Myers, 2003) and training induced improvement in $\dot{V}O_{2peak}$ is associated with a reduction in all-cause mortality risk (Lee et al., 2010). The improvements in $\dot{V}O_{2peak}$ following the Home-HIT intervention are therefore likely to be clinically meaningful.

Insulin dose and glucose concentrations were recorded during the first and final weeks of the training programme using an 8-point scale over 24h. In the sixth week of the Home-HIT programme there was a 13% reduction in insulin dose, indicating increased insulin sensitivity. This is important because insulin sensitivity is linked with decreased risk of cardiovascular complications in people with type 1 diabetes (Schauer et al., 2011, Bergman et al., 2012a). These results are in agreement with Salem et al. (2010) who also found significant effects of exercise training on insulin dose. We also recorded blood glucose levels using the same 8-point scale but showed no change in mean blood glucose concentration or daily glucose variation between weeks 1 and 6. However, the use of capillary glucose measures makes this difficult to interpret as there are many factors that could influence blood glucose concentrations between the time points. Future research should investigate the effects of Home-HIT on glycaemic control using continuous glucose monitors and HbA1c as these are the main clinical measures (Danne et al., 2017).

The survey responses support the previous findings of Brazeau et al. (2008) and Lascar et al. (2014) that fear of hypoglycaemia is the main barrier to exercise for people with type 1 diabetes. Therefore, development of strategies to reduce this barrier are critical to improve exercise participation. The training diary results show that blood glucose remained stable even up to 1h after the Home-HIT sessions (Fig. 8.2B), supporting the findings from a previous study in our laboratory (**Chapter 7**) that showed that blood glucose concentration did not change during laboratory-based HIT. This contrasts with MICT, which is recommended by the American

Diabetes Association (ADA), where there is a consistently large drop in blood glucose in people with type 1 diabetes (**Chapter 7**, (Garcia-Garcia et al., 2015, Davey et al., 2013b, McMahon et al., 2007). The blood glucose data in this study was supported by the survey responses, as participants said that they were comfortable doing Home-HIT at any time of day because they felt their blood glucose concentrations would remain stable. This emphasises the potential of HIT to reduce the major barrier of fear of hypoglycaemia which may increase exercise participation in people with type 1 diabetes. This is clinically relevant because Brazeau et al. (2008) found that individuals with greater perceived barriers to physical activity had poorer glycaemic control as measured by HbA1c. Although the data from this trial is promising, not all participants reported positive experiences, with one participant reporting that they felt their blood glucose was more difficult to manage. As such, future research should focus on understanding individual variation in blood glucose management during exercise, potentially leading to more personalised exercise prescriptions.

Fear of hypoglycaemia means people with type 1 diabetes regularly check their blood glucose concentration when performing traditional MICT recommended by the ADA (Colberg et al., 2016). The survey responses provide evidence that stopping to check blood glucose and correct accordingly with carbohydrate during exercise can be frustrating as it means that an already time consuming MICT session is even longer while they wait for their blood glucose to stabilise so they can recommence exercise. Despite the apparent importance of blood glucose correction during exercise this is the first study to quantify the number of training sessions during which participants had to prevent or treat an episode of hypoglycaemia through ingestion of carbohydrate. The participants in this investigation were able to complete every session without stopping to check their blood glucose concentration and out of the 188 Home-HIT sessions completed there were just 10 occasions

where participants had to ingest carbohydrate following exercise to prevent hypoglycaemia. This further highlights the time efficiency and ease of Home-HIT for people with type 1 diabetes. Importantly, the data on carbohydrate consumption and blood glucose concentrations during and after exercise would suggest that HIT may provide more stable blood glucose concentrations following exercise sessions, which may lead to better glycaemic control in the long term. Indeed, Kennedy et al. (2013) suggested that previous research may have failed to show glycaemic benefits of exercise because calorie intake and insulin dose around the time of exercise has not been controlled. The long term effects of Home-HIT on glycaemic control should therefore be investigated further.

Adherence and compliance to Home-HIT were high and comparable to that of our previous study investigating Home-HIT in obese individuals with elevated cardiovascular disease risk (**Chapter 4**). In fact, adherence was higher than two recent supervised field-based HIT interventions where participants attended pre-scheduled training sessions (Lunt et al., 2014, Shepherd et al., 2015). The responses to the survey strongly support our high adherence and compliance data suggesting that the Home-HIT programme was positively received, and 10 out of the 11 participants said that they would continue to use Home-HIT as a means to stay physically active after the study. Stable blood glucose values during the exercise may have contributed to the high adherence levels as there was no change in blood glucose concentration up to 1h post exercise and the survey responses were mostly positive regarding blood glucose levels. Another positive of Home-HIT is that it is time-efficient, as this has been highlighted as a major barrier to exercise here and in previous studies (Lascar et al., 2014, Brazeau et al., 2008). Training sessions lasted between 12 and 20 minutes meaning that weekly time commitment of Home-HIT is at least 90 minutes less than the 150 minutes that are recommended in the guidelines (Colberg et al., 2016). The results from the survey confirm this point, as

many people saw the time efficiency of HIT as a major advantage and liked the convenience of not having to travel. Other positive factors that were highlighted about exercising at home were that it helps with body image problems of exercising in front of others and the interval style exercises reduced boredom. The survey responses also highlighted that lack of motivation is often a key barrier to achieving physical activity guidelines. Having an exercise programme to follow with instant feedback from the HR monitor and mobile app was viewed positively. For example, one person said that they liked the feeling of someone (or something) monitoring them during exercise because it increased their motivation to complete all of the sessions. Two people said that especially when the training got to 10 intervals in weeks 5 and 6 that they may not have been motivated to do the exercise unless they felt they were being monitored.

The major strength of this study is the multi-disciplinary approach combining physiological data with qualitative evaluation of a training intervention. This is the first study to qualitatively assess the acceptability of an exercise-training programme in people with type 1 diabetes, and to the authors' knowledge only the third study to investigate the barriers to exercise in people with type 1 diabetes (Brazeau et al., 2008, Lascar et al., 2014). Inclusion of the survey allowed us to explore in more depth how effective Home-HIT was at overcoming perceived barriers to exercise and to provide contextual information for the physiological data. The current investigation was designed as a proof of concept study, and as such the sample size was small. However, the sample size is in line with other exercise studies that have been conducted in people with type 1 diabetes (Harmer et al., 2008) and the promising results strongly suggest that future trials should investigate Home-HIT in larger cohorts over longer time periods to investigate its effectiveness compared to traditional training interventions.

Chapter 8

The results of this pilot study demonstrate that Home-HIT is an effective intervention for people with type 1 diabetes resulting in high adherence to training that leads to increases in $\dot{V}O_{2peak}$ and reduced insulin dosage. Importantly Home-HIT did not reduce blood glucose concentrations and carbohydrate was not needed to correct blood glucose during exercise. This data in combination with qualitative feedback suggests Home-HIT is a safe strategy that, for most, reduces the major barriers including fear of hypoglycaemia and lack of time. We therefore suggest that Home-HIT is an effective strategy to increase exercise participation in people with type 1 diabetes.

Chapter 9 General Discussion

Chapter 9

9.1 Thesis Overview

We are currently experiencing a global obesity epidemic with >650 million adults worldwide classified as obese (BMI ≥30 $kg \cdot m^{-2}$), which is forecast to increase to 1.12 billion by 2030 (Kelly et al., 2008, World Health Organisation, 2018). The World Health Organisation has identified physical inactivity as one of the leading global risks for mortality, and obesity has been shown to double the risk of all-cause mortality due to its association with cardio-metabolic pathologies such as cardiovascular disease (CVD) and type 2 diabetes (Berrington de Gonzalez et al., 2010). The cost of prevention and treatment of chronic diseases resulting from inactivity in the UK has been estimated at a staggering £8.2 billion per year (both direct treatment and indirect costs e.g. sick absence), placing an enormous financial burden on the economy. Exercise prescription consisting of moderate-intensity continuous exercise, in line with the physical activity guidelines (Colberg et al., 2016), is an important first line strategy for the management of obesity and cardio-metabolic disease (Ismail et al., 2013). However, adherence to exercise programmes comprising continuous bouts of prolonged exercise is poor unless there is adequate supervision (Eriksson and Lindgarde, 1991, Faulkner et al., 2014).

Many people attribute their inactivity levels to a number of common exercise barriers including limited access to facilities and appropriate equipment, difficulty with transportation or inadequate financial resources (Korkiakangas et al., 2009) and the most commonly cited barrier, a “lack of time” (Trost et al., 2002). High-intensity interval training (HIT) protocols have been developed to reduce the time required to achieve health and fitness benefits, thus overcoming this “lack of time” barrier (Little et al., 2011, Weston et al., 2014). A plethora of supervised laboratory-based HIT programmes have shown clear improvements in numerous cardio-metabolic risk factors including aerobic capacity, insulin sensitivity and glycaemic control to a similar degree as moderate-intensity continuous training (MICT) (Cocks et al., 2013,

Cocks et al., 2016, Bradley et al., 2014, Wisloff et al., 2007, Tjonna et al., 2008). However, current evidence for the efficacy of HIT originates from researcher-led laboratory-based investigations using specialised equipment under close supervision to ensure correct training thresholds are achieved (Shepherd et al., 2017, Little et al., 2011, Gillen and Gibala, 2014, Weston et al., 2014). Furthermore, the applicability of current HIT programmes to the sedentary obese population has been disputed by public health experts (Biddle and Batterham, 2015, Hardcastle et al., 2014), who cite the strenuous nature and complex protocols as major barriers when targeted at sedentary, exercise-naïve individuals. Therefore, although time efficient, laboratory-based HIT does not address the additional barriers to exercise outlined above, meaning that the majority of the population are unlikely to adopt HIT as a strategy to improve their health and fitness.

The overall aim of this PhD was to provide evidence that practical HIT strategies can remove many of the major exercise barriers for obese individuals and people with type 1 diabetes, that could potentially be used to improve population health by increasing physical activity participation. Five studies were conducted that investigated the effects of HIT in these at-risk individuals. The physiological effectiveness of the HIT programmes, as well as adherence and compliance, were tested thoroughly throughout this thesis to provide a comprehensive evaluation of the exercise training programmes. **Chapter 4** aimed to eliminate many of the common exercise barriers by modifying existing HIT protocols to create a new Home-HIT intervention tailored to individuals with low fitness and mobility. In **Chapter 5**, muscle biopsies were analysed from the Home-HIT intervention to investigate the structural and endothelial enzymatic changes in skeletal muscle microvasculature in response to the 12-week training programme. In **Chapters 6**, **7** and **8** the focus shifted to people with type 1 diabetes, and the major barriers to exercise faced by this population of fear of hypoglycaemia and lack of time (Lascar

et al., 2014, Brazeau et al., 2008). **Chapter 6** investigated the effects of a single bout of HIT on glycaemic control under laboratory-controlled conditions in people with type 1 diabetes. **Chapter 7** then assessed the effects of six weeks of HIT on markers of cardio-metabolic health in people with type 1 diabetes. Finally, **Chapter 8** introduced Home-HIT to people with type 1 diabetes, as a potential strategy to reduce the remaining exercise barriers in this population.

9.2. Importance of Home-HIT

Since its development, many have touted HIT as the exercise mode that would increase exercise participation due to its effectiveness and time-saving potential in comparison to traditional MICT (Gibala et al., 2012, Weston et al., 2014, Gillen and Gibala, 2014). However, a large proportion of the population remain inactive suggesting further research is needed to develop more feasible strategies that can be adopted by the majority of the population. The overall aim of **Chapters 4** and **5** was to assess the physiological effectiveness of a novel Home-HIT programme tailored to obese individuals with low mobility and fitness that could be performed in their own home with no equipment. A wide range of physiological assessments were conducted including fasting blood lipid profile and oral glucose tolerance test, DXA scanning to assess body composition, aortic pulse wave velocity to assess vascular stiffness, flow-mediated dilation, $\dot{V}O_{2peak}$ and biopsies to assess skeletal muscle adaptations. This comprehensive approach allowed a full assessment of Home-HIT in comparison to the fully supervised laboratory-based HIT (Lab-HIT) and home-based MICT (Home-MICT) programmes. Altogether, the results of this pilot study provide robust evidence that Home-HIT is physiologically effective in obese individuals. The high adherence and compliance data in the Home-HIT group suggest that this sedentary obese group were able to maintain the programme for 12

weeks at the correct intensity without researcher supervision. Therefore, Home-HIT may represent a strategy to increase physical activity levels of the obese population.

Another important finding from the Home-HIT intervention was that the physiological benefits of HIT continue for at least 12 weeks. Previously, little was known about the long-term benefits of HIT compared to endurance training as most studies only investigated short-term training up to 6 weeks (Little et al., 2011, Gibala et al., 2012, Burgomaster et al., 2008). Although HIT is effective in the short-term, it was not known whether this would continue over a period of months. In their review on HIT, Gibala and McGee (2008) posed the question of whether the intense nature of interval training may stimulate relatively rapid physiological adaptations, whereas adaptations induced by traditional endurance training may take longer but progress for longer. The only study to date that has attempted to answer this was conducted by Gillen et al. (2016), who compared the cardio-metabolic effects of 12 weeks of sprint interval training (SIT) to endurance training in lean sedentary individuals and found similar improvements with both training modes. In **Chapter 4** the physiological adaptations to HIT were measured at weeks 4 and 12 to assess a time-course effect. $\dot{V}O_{2peak}$ increased significantly by 9% following 4 weeks of Home-HIT, and continued to increase by a further 7% between weeks 4 and 12, providing evidence that $\dot{V}O_{2peak}$ does not plateau for at least 12 weeks of training. Our data suggest that HIT, including Home-HIT, does have the potential as a long-term training strategy, at least in obese individuals with elevated CVD risk.

9.2.1. Myocyte Adaptations

In **Chapters 4** and **5** immunofluorescence microscopy was used to assess the effects of 12 weeks of Home-HIT on adaptations in skeletal muscle using biopsy samples taken pre- and post-training. This analysis aimed to provide mechanistic

insight into the training-induced improvements in $\dot{V}O_{2max}$ and insulin sensitivity. In **Chapter 4**, mitochondrial density, intramuscular triglyceride (IMTG) content and GLUT4 protein expression were assessed pre- and post-training, as these are classical markers of training adaptation. **Chapter 5** aimed to investigate the effect of the 12-week Home-HIT programme on skeletal muscle microvascular density and skeletal muscle microvascular enzymes responsible for NO bioavailability (eNOS content and ser^{1177} phosphorylation, NOX2 and $p47^{phox}$ content) in comparison to Home-MICT and supervised Lab-HIT.

Mitochondrial biogenesis is a major training adaptation that increases lipid and glucose fuel handling following exercise training, contributing to improvements in $\dot{V}O_{2peak}$ and insulin sensitivity (Holloszy, 1967). **Chapter 4** showed that Home-HIT increased mitochondrial content in both type 1 and 2 muscle fibres with no differences between the control training groups (Lab-HIT and Home-MICT). This suggests that Home-HIT is an effective means to increase mitochondrial content and supports previous research showing mitochondrial biogenesis in both fibre types with SIT (Shepherd et al., 2017) and HIT (Tan et al., 2018).

The increased central IMTG content and lipid droplet number found following Home-HIT is a training adaptation that has been seen in previous SIT interventions in lean (Shepherd et al., 2013a) and obese (Shepherd et al., 2017) individuals. This adaptation is purported to allow increased turnover of the IMTG pool because IMTG-containing lipid droplets have greater access to mitochondria for oxidation due to the increased surface area for interaction with lipases, suggesting improved metabolic flexibility and capacity for IMTG utilisation during exercise (Shepherd et al., 2017). There is a reorganisation of lipid droplets following training so that there is a higher fraction located in the intermyofibrillar space rather than the peripheral region. This appears to be an important adaptation for improved insulin sensitivity (Nielsen et al., 2017). It could be speculated that this adaptation will result in a reduction in the

accumulation of lipid metabolites such as ceramides, LCFA-CoA and diacylglycerol that can impair insulin signalling at the insulin receptor substrate-1 and Akt/PKB (Shulman, 2014, Amati et al., 2011, Shaw et al., 2010), eventually leading to improved insulin sensitivity.

The increase in GLUT4 expression likely contributed to the increased insulin sensitivity observed in **Chapter 4**. Increased GLUT4 protein expression was previously reported to increase following 6 weeks of MICT and SIT in lean sedentary males (Bradley et al., 2014). GLUT4 is the primary insulin-responsive glucose transporter in skeletal muscle, and experimental increases in skeletal muscle GLUT4 expression in animal models have been shown to increase whole-body insulin sensitivity (Tsao et al., 1996, Ren et al., 1995, Hansen et al., 1995).

Chapter 5 investigated the adaptations to the skeletal muscle microvasculature, as these are key mechanisms underpinning the increased insulin sensitivity and aerobic capacity. Obesity and sedentary behaviour lead to structural and functional impairments in skeletal muscle microvasculature that are associated with endothelial function loss and development of metabolic disease. **Chapter 5** demonstrated for the first time that 12 weeks of HIT in obese individuals with elevated CVD risk improves skeletal muscle capillarisation and the eNOS/NADPHox ratio of capillaries and arterioles, potentially improving skeletal muscle microvascular function. These data support previous work (Cocks et al., 2013, Cocks et al., 2016), that has shown exercise training to be effective at improving skeletal muscle microvascular health. **Chapter 5** is the first study to demonstrate that two home-based exercise interventions performed without supervision or equipment improve capillary density, capillary contacts and capillary-fibre perimeter exchange index to a similar degree to Lab-HIT. Increased capillarisation is an established adaptation to exercise training that contributes to increases in $\dot{V}O_{2max}$ and insulin sensitivity (Saltin et al., 1998, Akerstrom et al., 2014). Twelve weeks of Home-HIT also increased

eNOS content within the microvascular endothelium of arterioles and capillaries of skeletal muscle to a similar degree to Lab-HIT and Home-MICT, which is in agreement with other training studies that have been conducted in lean and obese individuals (Hoier et al., 2013, Cocks and Wagenmakers, 2016, Cocks et al., 2013).

Taken together, the immunofluorescence microscopy data in **Chapters 4** and **5** provide strong evidence that Home-HIT leads to improvements in a wide range of factors associated with insulin sensitivity and $\dot{V}O_{2max}$.

9.3. High-Intensity Interval Training is a Safe, Efficient and Effective Form of Exercise for People with Type 1 Diabetes

9.3.1. Glycaemic Effects of an Acute Bout of HIT and MICT

Before and after the exercise training sessions in **Chapters 6 and 7** blood glucose concentration was recorded for safety reasons to prevent participants from exercising when they were too high or low based on the Exercising for Type 1 Diabetes (EXTOD) guidelines (Narendran et al., 2017). In **Chapter 6**, exercise was undertaken in the fasted state where participants had omitted their short-acting insulin, whereas in **Chapter 7**, participants were in the fed state. Taken together, these data enable a comparison of the acute change in blood glucose from pre to post-exercise in the participants that completed HIT and MICT, as well blood glucose responses to exercise undertaken in the fasted (**Chapter 6**) and fed state (**Chapter 7**). From these comparisons, the most important observation was that HIT completely eliminated the fall in blood glucose concentration in both the fed and fasted state, removing the risk of acute hypoglycaemia (Figure 9.1). Blood glucose was also stable following MICT in the fasted state, but there was a consistently large drop of ~5.5 mmol/L following MICT in the fed state, with participants at risk of hypoglycaemia. These findings are clinically important because fear of

hypoglycaemia prevents many patients from taking part in exercise (Brazeau et al., 2008).

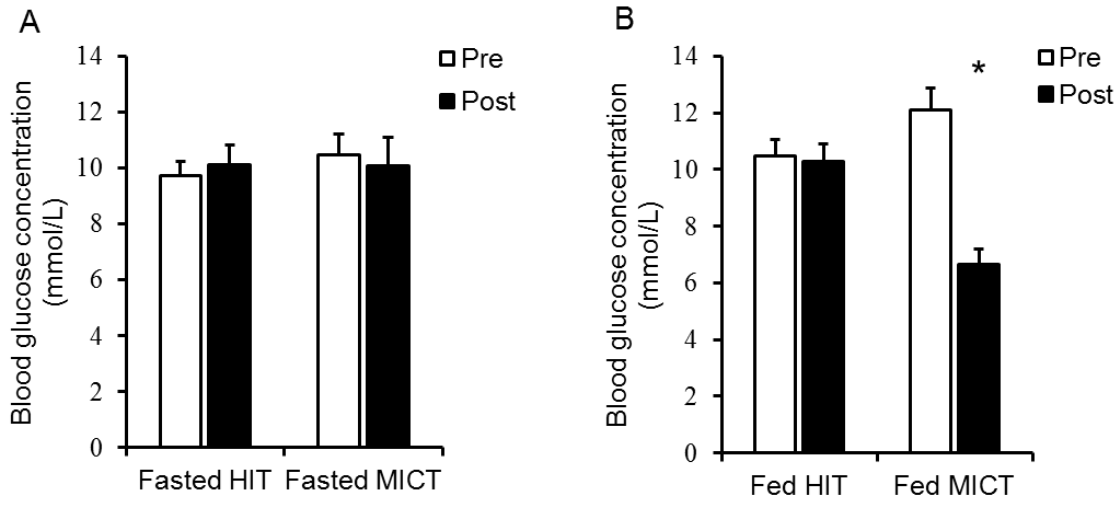

Figure 9.1. Change in blood glucose concentration following exercise in the fed or fasted state adapted from Chapters 4 and 5.

(A) Shows the mean change in blood glucose concentration following the HIT and MICT sessions where the participants were fasted and had omitted their fast acting insulin from Chapter 4. (B) Shows mean changes in blood glucose concentration during HIT or MICT over the 6-week training programme (Chapter 5) in the fed state. *Denotes a significant change from baseline (P<0.05).

The patterns reported here are striking and are the first of their kind in the literature over so many training sessions to compare blood glucose responses between the fed and fasted states. The high number of training sessions that were assessed demonstrates that the stability in blood glucose following HIT in both the fed and fasted state is consistent. Garcia-Garcia et al. (2015) conducted a systematic review and meta-analysis in which they aggregated results from 10 studies to estimate rate of change of blood glucose concentration during and after different types of exercise in people with type 1 diabetes. Their results showed a rapid decline in glycaemia during continuous exercise (-4.43 mmol/L h^{-1} on average) while there were more

variable results during intermittent high-intensity exercise, depending on the protocol. The findings from the studies presented here have important implications for people with type 1 diabetes who want to undertake exercise. The data suggest that people that want to perform MICT should try to do so in the morning before food and short-acting insulin, but there is more flexibility with HIT as it can be performed in the fed and fasted state without a significant reduction in blood glucose concentration.

In non-diabetic individuals, several counterregulatory mechanisms exist that occur in a stepwise and hierarchical fashion during moderate-intensity exercise that mean glucose uptake and production are precisely matched to maintain blood glucose concentration within a tight range of ~4-6 mmol/L (Cryer, 1997). At the onset of aerobic exercise in an individual without type 1 diabetes, endogenous insulin secretion is suppressed to below fasting levels via sympathetic innervation of the islets of Langerhans (Robertson et al., 1987). Concomitantly, there is an increase in glucagon secretion from the α islets in the pancreas into the portal vein which stimulates release of glucose from the liver, to match the rate of glucose uptake into the working muscles (Camacho et al., 2005). The decrease in insulin also sensitises the liver to glucagon which causes a rapid rise in cyclic AMP in the liver to stimulate glycogenolysis and gluconeogenesis (Vranic et al., 1976b, Vranic et al., 1976a, Zinker et al., 1994). Gluconeogenesis is crucial for preservation of hepatic glycogen stores and becomes increasingly important with intense, prolonged exercise. Other counter-regulatory hormones including adrenaline, growth hormone, cortisol, noradrenaline, aldosterone and adrenocorticotropic hormone are also released. These hormones stimulate hepatic glucose production and adipose tissue lipolysis, as well as inhibiting skeletal muscle glucose uptake in order to protect against hypoglycaemia (Marliss and Vranic, 2002).

However, as insulin is supplied exogenously in people with type 1 diabetes, hyperinsulinaemia is likely to occur during exercise because of increased blood flow

and mobilisation of insulin from its subcutaneous depot, particularly if the injection site is in an exercised region (Marliss and Vranic, 2002). This results in enhanced glucose uptake due to combined contraction-mediated and insulin-stimulated GLUT4 translocation. The high insulin levels will also suppress the exercise-mediated increases in glucagon and adrenaline and their ability to stimulate hepatic glucose production (Guelfi et al., 2007a). As a result, muscle glucose uptake during MICT in the fed state will exceed hepatic glucose production, leading to the large decreases in plasma glucose concentration observed in **Chapter 7**. Hyperinsulinaemia has also been shown to suppress adipose tissue and IMTG lipolysis in healthy individuals (Coyle et al., 1997), which will reduce the contribution of lipid metabolism during exercise. The combination of insulin and exercise-mediated glucose disposal coupled with decreased hepatic glucose production and reduced lipolysis and lipid oxidation increases the risk of hypoglycaemia during MICT in people with type 1 diabetes.

During high-intensity exercise circulating catecholamines increase 14-18 fold compared to only 2-4 fold during moderate-intensity exercise. This increase in catecholamines is a strong stimulus for glycogenolysis, inducing a 7-8 fold increase in hepatic glucose production (Marliss and Vranic, 2002). Interestingly, Kreisman et al. (2003) showed that infusion of adrenaline and noradrenaline in healthy non-diabetic males during moderate-intensity exercise resulted in augmented hepatic glucose output to the same magnitude as during intense exercise. Moreover, an increase in circulating catecholamines enhances glucose rate of appearance even in the presence of hyperinsulinaemia (Bally et al., 2016). This suggests that catecholamines are important in the regulation of glucose homeostasis and could explain the improved glycaemic stability during HIT in people with type 1 diabetes even in the fed state where they have injected insulin. Indeed, circulating insulin does not decrease as markedly during high intensity exercise in people without type 1 diabetes, partly because the duration of the activity is typically shorter (Marliss and

Vranic, 2002). Increased epinephrine also inhibits muscle glucose uptake during exercise (Watt et al., 2001), which appears to be due to its stimulatory effect on muscle glycogenolysis and the subsequent increase in muscle glucose-6-phosphate (an inhibitor of hexokinase and glucose phosphorylation).

Following a high-intensity exercise bout in non-diabetics, there is a small hyperglycaemic response that persists for up to an hour (Marliss and Vranic, 2002). Insulin secretion increases post-exercise to normalise the hyperglycaemia and to permit rapid recovery of muscle glycogen (Marliss and Vranic, 2002). The issue in those with type 1 diabetes is that the inability to increase endogenous insulin in early recovery of high intensity exercise can result in greater or more sustained hyperglycaemia, possibly increasing the risk of ketoacidosis or hyperglycaemia related complications. Figure 9.2 provides information about the hormonal responses before, during and after high and moderate intensity exercise in healthy individuals without type 1 diabetes.

Growth hormone and cortisol secretion also appear to play a minor role in the regulation of glucose homeostasis during exercise, albeit indirectly, as they contribute to stimulation of whole-body lipolysis. In normal, healthy individuals, carbohydrate ingestion during prolonged moderate intensity exercise supresses cortisol secretion (Deuster et al., 1992). In addition, carbohydrate ingestion immediately before and during the first hour of prolonged running has been shown to attenuate the normal increase in growth hormone alongside suppression of lipolysis (Tsintzas et al., 1996). These studies suggest that secretion of growth hormone is important for fatty acid mobilisation from adipose tissue and therefore indirectly for glucose metabolism. During MICT in the fasted state, there will be greater lipolysis than the fed state which would contribute to more stable blood glucose, as observed in **Chapter 6**.

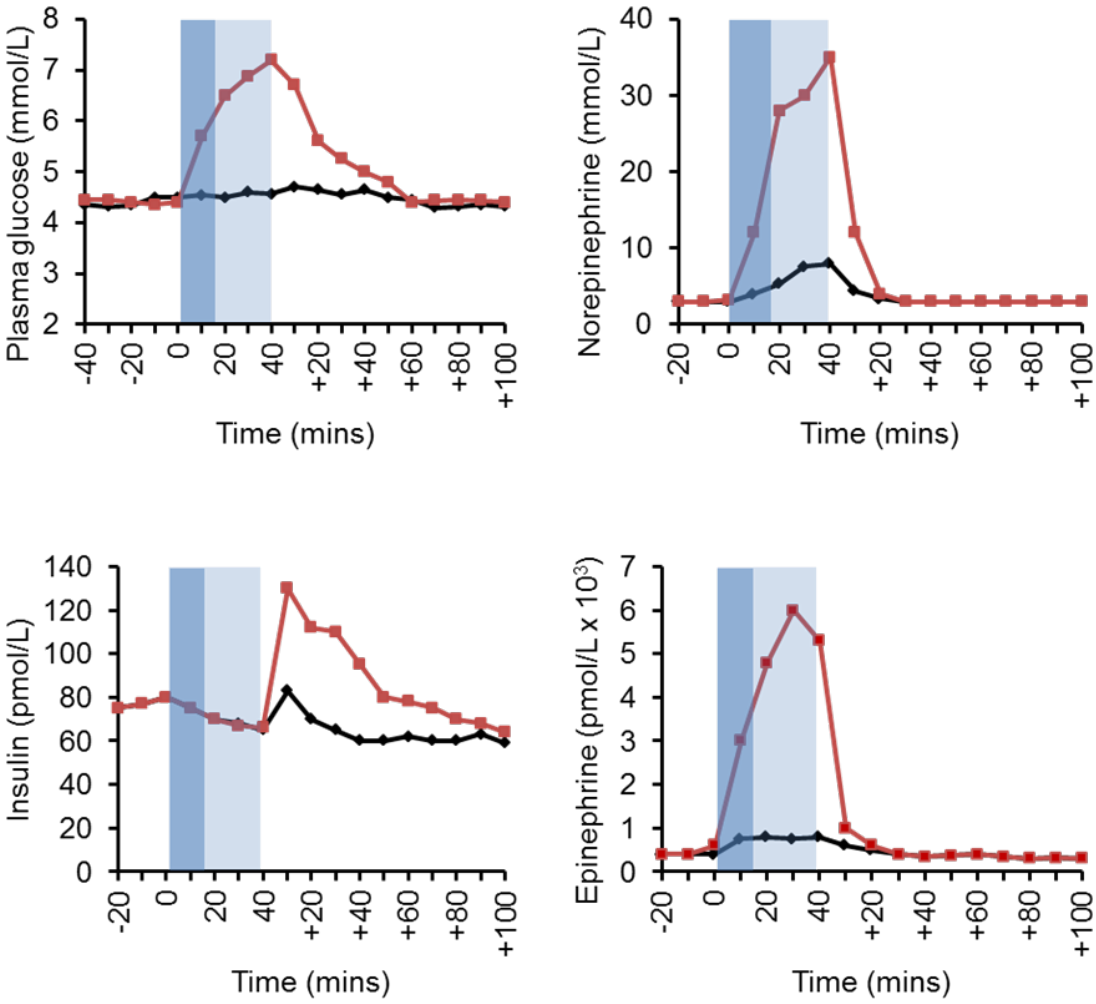

Figure 9.2. Hormonal responses to high and moderate intensity exercise

Comparison of plasma glucose and hormonal responses during 40 minutes of moderate intensity exercise (50% $\dot{V}O_{2max}$; black) and 15 minutes of high intensity exercise (87% $\dot{V}O_{2max}$; red) in healthy non-diabetic males. A rest period was followed by exercise at either high intensity (dark shading) or moderate intensity (light shading). Adapted from Marliss and Vranic (2002).

Bally et al. (2016) aimed to investigate the metabolic and hormonal response to intermittent high-intensity exercise and continuous exercise in people with type 1 diabetes. They used stable isotope techniques, non-invasive measurement of liver glycogen (magnetic resonance spectroscopy) and assessment of whole-body substrate utilisation during a 90-minute cycle at 50% $\dot{V}O_{2max}$ either with or without 10-

second sprint every 10 minutes. Glucose requirements were lower during the intermittent high-intensity exercise condition compared with continuous exercise alone which paralleled an increase in counterregulatory hormones and lactate. Unexpectedly, the lower glucose requirements during the last 30 minutes of exercise of the intermittent sprint condition were due to lower glucose disposal rather than an increase in hepatic glucose output. The higher lactate levels in the intermittent exercise suggest that there may be a shift to consumption of alternative substrates that reduce glucose disposal and therefore help maintain blood glucose levels. One limitation of our HIT studies (**Chapters 6** and **7**) is that we did not measure catecholamines, lactate or growth hormone concentrations which would help with interpretation of the results. Differences in lipid oxidation may also account for the differences in glycaemic response between HIT and MICT as a greater catecholamine response during HIT may trigger IMTG utilisation due to stimulation of hormone sensitive lipase (Watt et al., 2003).

Another important observation in **Chapters 6** and **7**, although not recorded, was the number of training sessions in which participants had to prevent or treat an episode of hypoglycaemia by consuming fast-acting carbohydrate. During most of the MICT sessions, especially the 50-minute sessions, participants had to stop exercising at least once to check their blood glucose, correct accordingly with glucose, and then wait for their blood glucose concentration to stabilise before recommencing the training. However, all participants were able to complete the HIT sessions without interruption. Many of the participants in the MICT condition found this frustrating and it would often mean that the already time-consuming 50-minute cycling sessions were even longer while they checked their blood glucose. This further highlights the time-efficiency and ease of HIT for people with type 1 diabetes. Furthermore, the carbohydrates used to prevent hypoglycaemia are high in calories which may make it difficult for patients to manage their weight. This can be especially off-putting for people wanting to exercise to lose weight, which is a

common goal (highlighted in **Chapter 8**). Secondly, taking on carbohydrate during or after the session is likely to cause their blood glucose to rise rapidly meaning they would be more likely to have to increase their insulin dosage to prevent hyperglycaemia later on, making it more difficult to control and predict blood glucose after the exercise session. Because blood glucose remains stable during HIT, it reduces the need to take on corrective carbohydrate or insulin, making blood glucose concentrations more predictable.

Based on the findings in **Chapters 6** and **7**, the protocol for **Chapter 8** was designed so that blood glucose responses and insulin dose around exercise were recorded in greater detail. In **Chapter 8**, participants were asked to record their blood glucose concentrations immediately pre, post and 1 hour post training over the 6-week training period so that change in blood glucose could be monitored over the 18 sessions. Participants were also asked to record whether they needed to take additional carbohydrates or insulin during or following the session to adjust their blood glucose. In addition, during the first and final 7 days of the training programme, participants were asked to monitor their blood glucose and insulin doses using an 8-point profile: before and 2 hours after each meal, just before bed, and at 2am. This was designed to provide information on whether the training programme had an effect on insulin dosage and glucose control over the week.

The results from **Chapter 8** support those of **Chapters 6** and **7**. Blood glucose remained stable during Home-HIT and 1 hour post-exercise and there was a low number of sessions that needed to be corrected with carbohydrate or insulin suggesting that HIT, specifically Home-HIT, does not increase the risk of acute hypoglycaemia. These results were supported by the qualitative survey responses, as a number of participants reported that they felt the Home-HIT exercises helped with blood glucose management. Overall, **Chapters 6**, **7** and **8** provide strong, novel

evidence that HIT in both the fed and fasted state abolishes the drop in glycaemia that is associated with MICT.

9.3.2. Effects of HIT on Markers of Cardio-Metabolic Health in People with Type 1 Diabetes

The findings from **Chapter 6** suggested that there is no greater risk of hypoglycaemia following HIT in comparison to a control day with no exercise, suggesting this training mode should be explored further. **Chapter 7** tested whether six weeks of HIT improves markers of metabolic health, including $\dot{V}O_{2peak}$, glycaemic control and vascular health, in people with type 1 diabetes in comparison to MICT. Six weeks of HIT led to comparable improvements in $\dot{V}O_{2peak}$ and arterial stiffness to MICT while being a time-efficient alternative that reduces the risk of acute hypoglycaemia. The two studies combined provide evidence that HIT is a safe, efficient and effective form of exercise for people with type 1 diabetes.

As discussed in **Chapter 7**, $\dot{V}O_{2peak}$ and arterial stiffness improved with training. However, there was no change in fasting plasma cholesterol or triglycerides, blood pressure, or blood glucose control indicated by time in target range from the continuous glucose monitoring systems (CGMS). This suggests that longer term training programmes are needed to see effects in these variables. A potential limitation of **Chapter 7** was that only one 24-hour CGMS period was recorded pre- and post-training to assess the effects of training on glycaemic control. At the time of study design, this single 24-hour period was in line with other studies that have used CGMS to assess the effects of exercise on glycaemic variables in people with type 1 and 2 diabetes (Little et al., 2011, Maran et al., 2010, Yardley et al., 2012, van Dijk et al., 2013). However, after data was collected for **Chapters 6** and **7**, an international consensus on use of continuous glucose monitoring was published (Danne et al., 2017) that provides clear criteria for collecting and analysing CGMS data for research. These criteria were used for the analysis of the data for **Chapters 6** and **7**

in regards to definition of glycaemic thresholds. However, the guidelines recommend that a minimum of 14 consecutive days of data are required to generate a report that enables optimal analysis, which is clearly much longer than the single 24-hour period analysed for the work in this thesis. The longer period of analysis would increase the reliability of the data and allow more information on individual variability, as although diet was controlled, there are many other factors that affect blood glucose concentration in people with type 1 diabetes (Riddell et al., 2017).

9.4. Directions for Future Research

9.4.1. Transferring Home-HIT into the 'Real World'

The Home-HIT investigation (**Chapter 4)** was designed as a proof of concept study, and as such the sample size was too small to detect differences in responses between the training modes. Nonetheless, this promising pilot study provides strong evidence that our novel Home-HIT programme offers an effective strategy to improve physical activity participation in obese individuals with elevated CVD risk. The data strongly suggest that the Home-HIT protocol elicits significant improvements in a wide range of cardio-metabolic health markers, and therefore should be explored further using larger cohorts. The use of a novel heart rate monitor and mobile phone app system was an important addition that allowed objective measurement of whether participants completed the exercises at the correct intensity. The high adherence and compliance data would certainly support this. This was further supported by **Chapter 8**, as there were high adherence and compliance results in the people with type 1 diabetes.

At present, a number of UK councils offer exercise referral schemes for individuals that meet certain criteria (e.g. inactivity, obesity, risk of CVD) and are deemed likely to benefit from regular exercise by their general practitioner. For

example, the Liverpool Clinical Commissioning Group offers a 12-week programme whereby individuals are given gym access with a fitness instructor available to offer support and guidance. Unfortunately, these schemes are not suitable for many and there are high levels of dropout (Kelly et al., 2017). A number of the barriers to exercise remain because the individual still has to travel to the 'intimidating' gym environment and exercise sessions are time consuming meaning that lack of time remains as a barrier (Morgan et al., 2016). Therefore, a large-scale randomised controlled trial should be conducted to test the effectiveness of Home-HIT vs. the current exercise referral scheme. It would also be useful to include a 3-month follow-up assessment after the intervention has finished, whereby physical activity, aerobic capacity, CVD risk factors, body composition and psychological well-being are reassessed. This study would provide evidence of whether Home-HIT can be transferred to the 'real world'. Further information is also required to develop the progression of Home-HIT over the long term so that individuals can continue to achieve health benefits even years after the start of a programme. Researchers will therefore have to consider ways in which motivation can be maintained for the long term so that high compliance rates are maintained while making the sessions challenging and achievable. Beyond the 12 weeks, individuals are unlikely to want to increase the number of intervals beyond 10, as the training sessions will lose their time saving advantage over MICT. Therefore, individuals will look to increase the number of sessions per week, alter the exercises that they are doing or add additional forms of training to vary their workouts.

A parallel large randomised controlled trial in people with type 1 diabetes to test the effects of Home-HIT against traditional care also seems appropriate. The findings from **Chapter 8** suggest that 6 weeks of Home-HIT is an effective strategy to improve fitness and reduce insulin requirements. A longer Home-HIT intervention, lasting a minimum of three months, is now required in order to assess changes in

glycaemic control and HbA1c. Combining a long-term assessment of glycaemic control using CGMS as per the most recent guidelines (Danne et al., 2017) before and after a training intervention alongside a series of markers of cardio-metabolic health would provide strong evidence for Home-HIT in people with type 1 diabetes.

Home-HIT may be particularly useful for people that have been recently diagnosed with type 1 diabetes. Kennedy et al. (2018) found that additional barriers to exercise tend to exist in newly diagnosed patients with type 1 diabetes. Around half of the people that took part in the investigation by Kennedy et al. (2018) reported a decline in their activity levels around the time of diagnosis. Many people in the study reported that there was a scarcity of information on blood glucose management around exercise. The findings in the study by Kennedy et al. (2018) suggest that a programme such as Home-HIT where individuals are given a blood glucose diary to complete alongside a training programme designed to reduce the major barriers to exercise may be particularly beneficial for people that are newly diagnosed with type 1 diabetes, as it may provide a supportive and educational programme that can be used to help the individual to better understand how exercise affects their blood glucose.

9.4.2. Effects of Training on Insulin-Stimulated Microvascular Perfusion

Increases in skeletal muscle microvascular blood flow that are seen in response to insulin infusion or a mixed meal are impaired in obesity (Keske et al., 2009, Clerk et al., 2006). The results from **Chapter 5** and the study by Cocks et al. (2016) suggest that with exercise training there is an increase in endothelial eNOS content of the microvasculature in obese individuals which presumably would lead to increased capillary perfusion following insulin stimulation. A limitation of these investigations is that the fasted muscle biopsy data do not provide insight into the regulation of microvascular perfusion under postprandial conditions. It would be assumed that

with training there would be greater eNOS ser^{1177} phosphorylation following insulin stimulation however this has not been tested in humans. Therefore, it would be important to investigate the effects of exercise training on the insulin-stimulated endothelial response. This would provide insight into the functional relevance of the observed changes in the microvascular enzymes measured in **Chapter 5**, the impairments in obesity and the adaptations with training.

In a previous investigation, Rattigan et al. (2001) found that endurance training in previously sedentary rats led to parallel increases in skeletal muscle microvascular perfusion and glucose uptake during a hyperinsulinaemic-euglycaemic clamp as measured using 1-methylxanthine clearance. However, to date this has not been done in humans. Future studies should use insulin clamps alongside contrast enhanced ultrasound (CEU) to measure microvascular recruitment. This could be done alongside the immunofluorescence techniques used in **Chapter 5** to investigate insulin-induced activation of eNOS. CEU has previously been used to measure microvascular blood volume and microvascular blood flow in response to insulin stimulation (Vincent et al., 2006, Vincent et al., 2004). CEU involves infusion of ~8 μm microspheres filled with contrast agent which can be visualised using ultrasound. The acoustic intensity of the image is proportional to the concentration of microspheres within the volume of tissue being measured. A single pulse of ultrasound is administered to destroy all of the microspheres within the ultrasound beam and then the rate of microsphere replenishment reflects the microvascular flow velocity and the plateau level of microspheres reached after destruction reflects microvascular blood volume. CEU may allow the rate assessment of how changes in microvascular eNOS content and insulin stimulated ser^{1177} phosphorylation lead to increased muscle microvascular perfusion and microvascular blood volume with training.

A further idea would be to investigate the translocation of GLUT4 following insulin stimulation in obese individuals pre- and post-training. Previously, Bradley et al. (2015) used immunofluorescence microscopy to show that there was an increase in the number of large GLUT4 spots at the plasma membrane layer of myocytes following glucose ingestion in healthy lean males, suggesting increased GLUT4 translocation. If glucose-stimulated samples were taken pre- and post-training, immunofluorescence microscopy could be used to assess the effects of training on GLUT4 translocation to provide further insight into the mechanisms of insulin resistance with obesity and inactivity.

9.4.3. Mechanisms of Blood Glucose Response to Fed vs. Fasted Exercise of Differing Intensities in People with Type 1 Diabetes

The protocol for **Chapter 6** was designed so that participants attended the laboratory under fasted conditions before taking their short-acting insulin to allow maximum control. The findings that HIT and MICT in the fasted state do not increase the risk of post-exercise hypoglycaemia over the 24-hour period and overnight are important. The current exercise guidelines do not advocate fasted exercise as a strategy for people with type 1 diabetes. However, the findings presented here suggest that provided appropriate precautions are taken it is safe for people with type 1 diabetes to exercise fasted.

The question that remains following **Chapter 6** is whether there is an increased risk of nocturnal hypoglycaemia when HIT is performed later in the day in both the fed and fasted states. Future research should investigate the effects of HIT during differing nutritional states and at different times of day in people with type 1 diabetes. It also remains to be determined whether longer duration MICT sessions are associated with a risk of hypoglycaemia as many people would wish to exercise

longer than 30 minutes. Furthermore, more research is needed to determine whether there is a long-term benefit of exercising in the fasted state on glycaemic control. Exercise strategies that produce more stable blood glucose levels may lead to long term improvements in glycaemic control determined by HbA1c and CGMS.

It is important to determine the mechanisms underpinning the maintenance of blood glucose levels during HIT, as this will help provide information on nutritional and insulin requirements. Investigating changes in hormones such as catecholamines, growth hormone and cortisol is a good starting point. For example, Marliss and Vranic (2002) assessed the hormonal profiles of high intensity and moderate intensity exercise in healthy individuals to provide insight into blood glucose response in people with type 1 diabetes (Figure 9.2). Muscle biopsies could be taken to provide information on muscle glycogen and IMTG utilisation during exercise. The use of glucose stable isotope tracers would enable investigation of rates of endogenous glucose production (rate of appearance), glucose uptake into peripheral tissues (rate of disappearance) and glucose oxidation during exercise. This work would be particularly useful if combined with magnetic resonance spectroscopy to study metabolic changes in the liver. In a comprehensive study, Bally et al. (2016) conducted a randomised cross-over study in which 12 individuals with well controlled type 1 diabetes completed a 90-minute cycle at 50% $\dot{V}O_{2max}$ either with 10 second sprints interspersed every 10 minutes or without. ^{13}C Magnetic resonance spectroscopy was used to quantify hepatocellular and intramyocellular glycogen during the exercise bouts. They also measured glucose kinetics using stable isotopes, hormones and metabolites. Bally et al. (2016) found that exogenous glucose requirements were significantly lower in the final 30 minutes of the intermittent exercise condition but this was not due to a difference in hepatic glucose output or glycogen utilisation. Instead, there was decreased glucose uptake which implied a shift towards alternative substrates due to the intermittent sprints. A similar,

comprehensive study using the HIT protocol employed in **Chapter 6** would provide detailed information on the metabolic responses to HIT in people with type 1 diabetes.

9.4.4. Myocyte Adaptations to Exercise Training in People with Type 1 Diabetes

Current understanding of the long-term impact of type 1 diabetes on the health and quality of human skeletal muscle is limited, and little is known about the effects of exercise training on myocyte adaptations in this population. A recent study by Monaco et al. (2018) provided the first assessment of skeletal muscle mitochondrial ultrastructure and bioenergetics in young adults with type 1 diabetes that met the physical activity guidelines. Their findings highlighted mitochondrial and autophagic differences within the muscles of young adults with type 1 diabetes and non-diabetic controls matched for age, sex, BMI and levels of physical activity. Transmission electron microscopy revealed that type 1 diabetes negatively affects skeletal muscle ultrastructure as shown by disorganised mitochondrial cristae and an increased presence of autophagic remants. Monaco et al. (2018) also observed a 20% reduction in mitochondrial oxidative capacity. These impairments in mitochondria are clinically important as skeletal muscle is the major site of fatty acid catabolism (Hargreaves, 2000), a key mediator of whole-body glucose homeostasis and a major determinant of whole-body insulin sensitivity (Jensen et al., 1997, Shulman et al., 1990). Therefore, impairments in skeletal muscle quality could have important long-term consequences to the development in diabetic complications (Soedamah-Muthu et al., 2006, Kilpatrick et al., 2007).

A major clinical concern highlighted by Monaco et al. (2018) was that the mitochondrial alterations that they observed were in young adults with type 1 diabetes that met the American Diabetes Association physical activity guidelines of

150 minutes of moderate-intensity exercise per week (Colberg et al., 2016). Furthermore, in an earlier investigation, Perseghin et al. (2003) showed that people with type 1 diabetes had higher IMTG content than controls without type 1 diabetes and this was associated with degree of glycaemic control. Therefore, investigation of the effects of exercise training on mitochondrial biogenesis and IMTG content in people with type 1 diabetes, perhaps using the immunofluorescence microscopy techniques described in **Chapters 4** and **5** of this thesis, may be important to determine optimal strategies to improve these markers and reduce the risk of insulin resistance. Future research should investigate the myocyte characteristics of sedentary and active people with type 1 diabetes, as well as the effects of chronic exercise training to provide insight into the adaptions with exercise. This will help to develop optimal exercise training strategies for people with type 1 diabetes to reduce the risk of metabolic complications.

9.5. Final Conclusions

The work conducted over the course of this PhD provides strong evidence for the use of Home-HIT as a strategy to remove the major barriers to exercise in sedentary populations that in the future may increase exercise participation and therefore population health. **Chapters 4** and **5** provide evidence that a novel Home-HIT protocol improves cardio-metabolic health and removes barriers to exercise in obese individuals with elevated CVD risk. The comprehensive range of physiological measures including blood analysis, body composition measures and muscle biopsies alongside the novel HR monitoring system provide clear evidence for the effectiveness. **Chapters 6** and **7** demonstrate that HIT is a safe and effective training strategy for people with type 1 diabetes that removes the major barriers of lack of time and fear of hypoglycaemia. This is especially important as this population is at increased risk of CVD and a high proportion of this population fail to reach physical

activity guidelines. Finally, **Chapter 8** successfully introduced Home-HIT to people with type 1 diabetes. Therefore, Home-HIT appears to be an effective and feasible exercise strategy to improve markers of cardio-metabolic health in the obese sedentary population and people with type 1 diabetes.

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Chapter 11 Appendix

HIT-it

High intensity interval training at home study

LIVERPOOL JOHN MOORES UNIVERSITY

HIT Training Diary

Name .....

Subject ID .....

A research project funded by The Physiological Society

Outline of Training Procedures

Home Based High Intensity Interval

Outline of the training program:

You should train 3 time per week. These sessions can be on any day of the week, but we advise a days rest between sessions. You can complete each session wherever you want.

Outline of the exercise session:

1. Choose the exercises you will complete before the session starts (see page 3-5 for exercise instructions.)
2. Put your heart rate monitor on and start to record.
3. Start with a 2-3 minute warm-up of jogging on the spot.
4. Do your first interval. This should consist of 30 seconds of the first exercise (e.g. T-Jumps) then 30 seconds of the second exercise (e.g. squat Touches). These should follow one another immediately.
5. Rest for 1 minute
6. Do the second interval
7. Rest for 1 minute
8. Repeat until the number of intervals required is completed.
9. Stop your heart rate monitor recording
10. Record what exercises you did and any notes of importance.

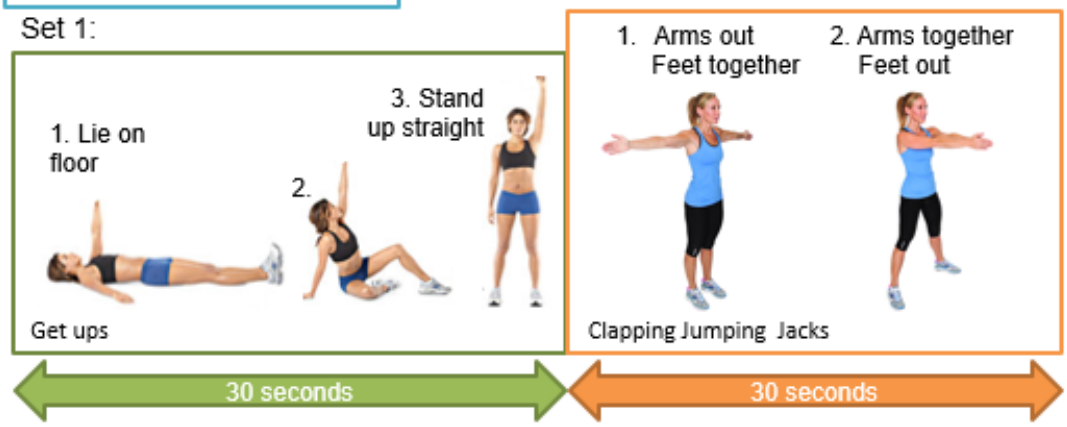
During each interval you should aim to achieve a heart rate equal to/ above your heart rate goal.

Your max heart rate = ... (=220-age)

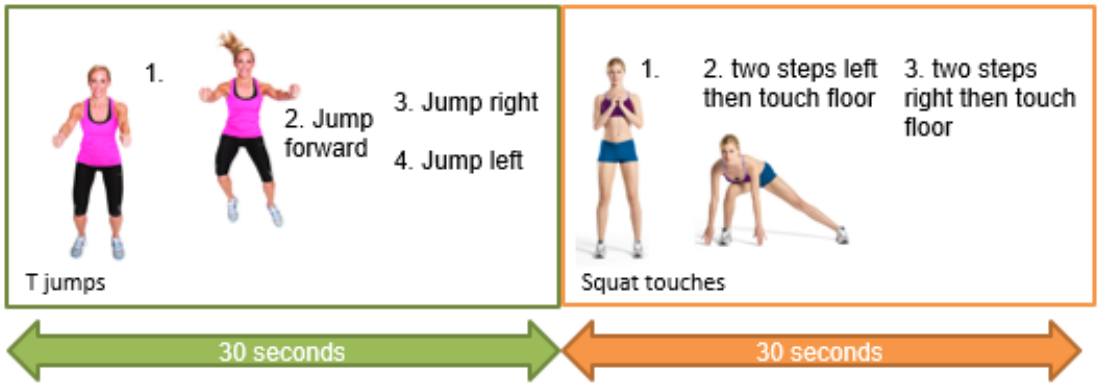
Your target heart rate = ... (80% of max)

Lower impact exercises

Set 1:

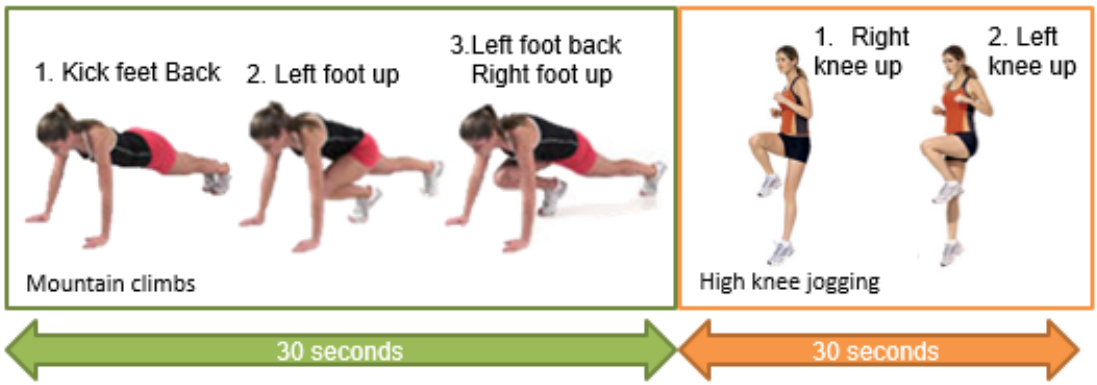


Set 2:

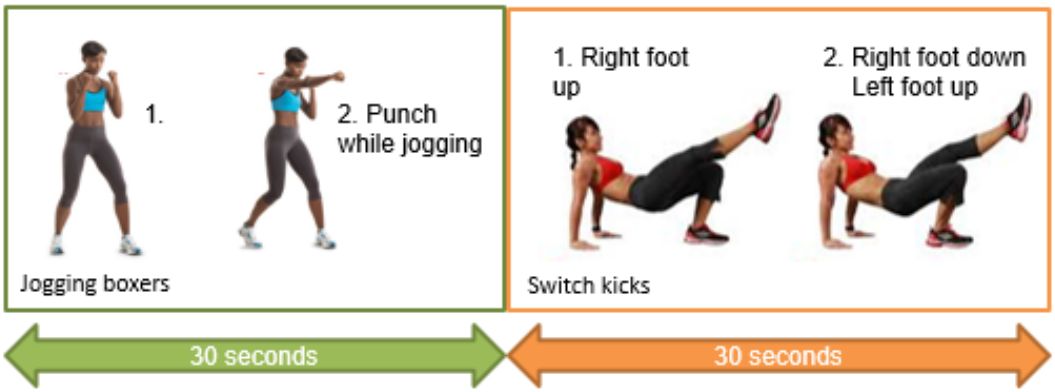


Moderate impact exercises

Set 3:

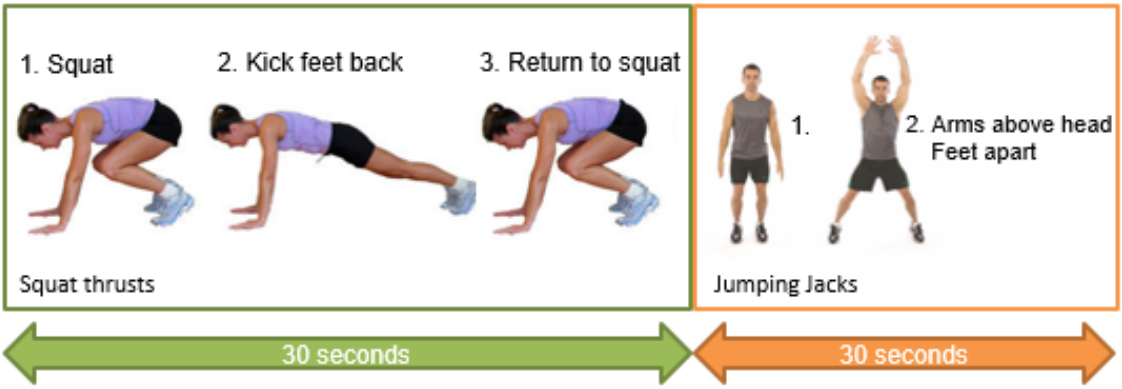


Set 4:

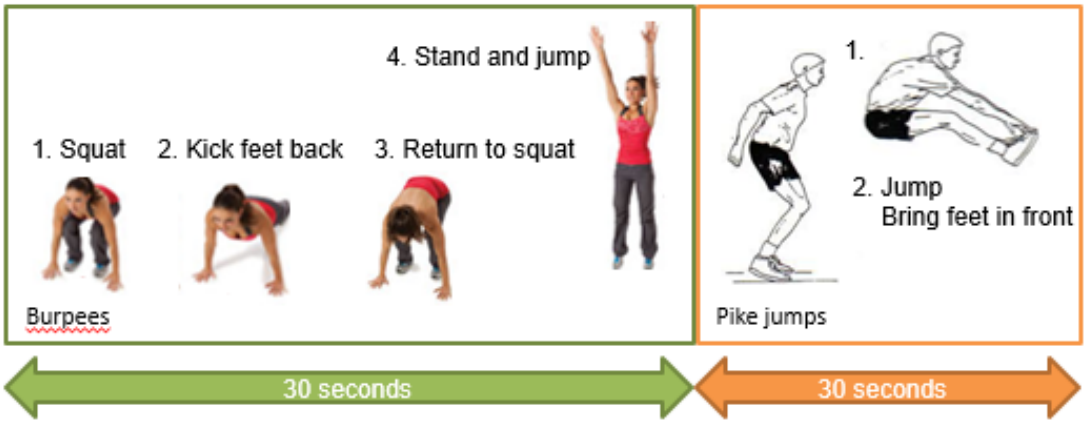


Higher Impact Exercises

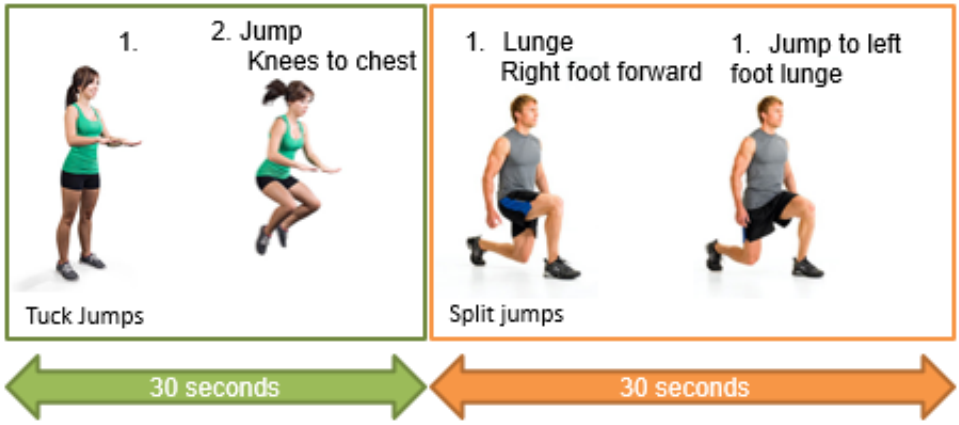
Set 5:



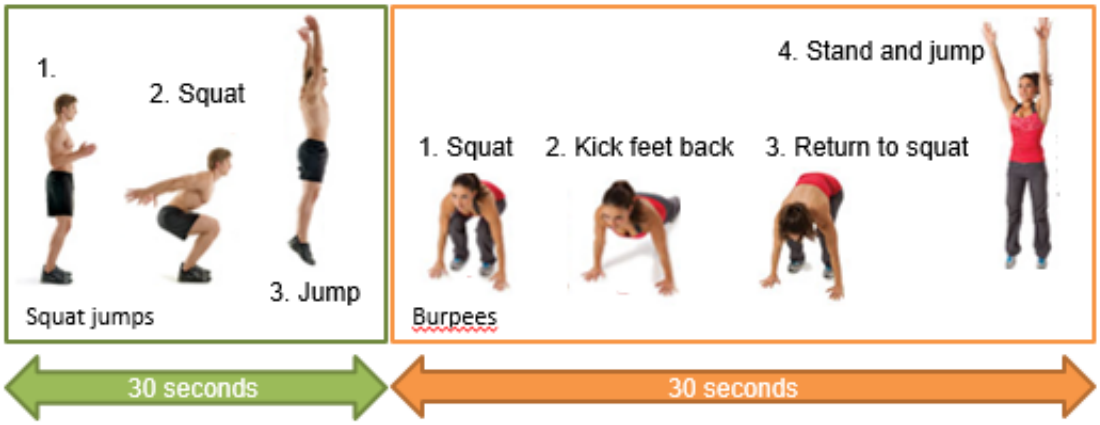
Set 6:



Set 7:


1.
2. Jump
Knees to chest
Tuck Jumps
1. Lunge
Right foot forward
1. Jump to left
foot lunge
Split jumps
30 seconds
30 seconds

Set 8:


1.
2. Squat
3. Jump
Squat jumps
4. Stand and jump
1. Squat
2. Kick feet back
3. Return to squat
Burpees
30 seconds
30 seconds

Training diary

Week 1								
Session 1	Interval 1	Interval 2	Interval 3	Interval 4				
Exercise								
Notes								
Session 2	Interval 1	Interval 2	Interval 3	Interval 4				
Exercise								
Notes								
Session 3	Interval 1	Interval 2	Interval 3	Interval 4				
Exercise								
Notes								
Week 2								
Session 4	Interval 1	Interval 2	Interval 3	Interval 4				
Exercise								
Notes								

Training diary

Session 5	Interval 1	Interval 2	Interval 3	Interval 4				
Exercise								
Notes								
Session 6	**Interval 1**	**Interval 2**	**Interval 3**	**Interval 4**				
Exercise								
Notes								
Week 3								
Session 7	**Interval 1**	**Interval 2**	**Interval 3**	**Interval 4**				
Exercise								
Notes								
Session 8	**Interval 1**	**Interval 2**	**Interval 3**	**Interval 4**				
Exercise								
Notes								

Training diary

Session 9	Interval 1	Interval 2	Interval 3	Interval 4				
Exercise								
Notes								
Week 4								
Session 10	Interval 1	Interval 2	Interval 3	Interval 4				
Exercise								
Notes								
Session 11	Interval 1	Interval 2	Interval 3	Interval 4				
Exercise								
Notes								
Session 12	Interval 1	Interval 2	Interval 3	Interval 4				
Exercise								
Notes								

Training diary

Week 5								
Session 13	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5			
Exercise								
Notes								
Session 14	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5			
Exercise								
Notes								
Session 15	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5			
Exercise								
Notes								
Week 6								
Session 16	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5			
Exercise								
Notes								

Training diary

Session 17	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5			
Exercise								
Notes								
Session 18	**Interval 1**	**Interval 2**	**Interval 3**	**Interval 4**	**Interval 5**			
Exercise								
Notes								
Week 7								
Session 19	**Interval 1**	**Interval 2**	**Interval 3**	**Interval 4**	**Interval 5**	**Interval 6**		
Exercise								
Notes								
Session 20	**Interval 1**	**Interval 2**	**Interval 3**	**Interval 4**	**Interval 5**	**Interval 6**		
Exercise								
Notes								

Training diary

Session 21	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6		
Exercise								
Notes								

Week 8

Session 22	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6		
Exercise								
Notes								

Session 23	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6		
Exercise								
Notes								

Session 24	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6		
Exercise								
Notes								

Training diary

Week 9								
Session 25	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	
Exercise								
Notes								
Session 26	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	
Exercise								
Notes								
Session 27	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	
Exercise								
Notes								
Week 10								
Session 28	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	
Exercise								
Notes								

Training diary

Session 29	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	
Exercise								
Notes								
Session 30	**Interval 1**	**Interval 2**	**Interval 3**	**Interval 4**	**Interval 5**	**Interval 6**	**Interval 7**	
Exercise								
Notes								

Week 11

Session 31	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8
Exercise								
Notes								
Session 32	**Interval 1**	**Interval 2**	**Interval 3**	**Interval 4**	**Interval 5**	**Interval 6**	**Interval 7**	**Interval 8**
Exercise								
Notes								

Training diary

Session 33	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8
Exercise								
Notes								

Week 12

Session 34	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8
Exercise								
Notes								

Session 35	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8
Exercise								
Notes								

Session 36	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8
Exercise								
Notes								