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Evaluation of lifestyle modification on cardiometabolic risk markers in overweight post-menopausal women: a ghrelin-mediated response?

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Abstract

Oestrogen deficiency in post-menopausal women increases their susceptibility to abdominal obesity, which can be associated with dysregulated ghrelin and cardiometabolic dysfunction. While a healthy lifestyle involving both regular physical activity and a healthy diet is important in managing cardiometabolic risks, understanding ghrelin's role may aid in optimising the synergistic effects of these behaviours in post-menopausal women. This thesis investigated the cardiometabolic response to home-based lifestyle modifications, while exploring ghrelin's mediatory role in physically inactive, overweight/obese post-menopausal women. Through a meta-analysis of published studies, the first project detailed the cardiometabolic benefits of mixed intensity exercise training of at least 8 weeks in post-menopausal women on their usual diet. The next study demonstrated that an 8-week home-based, equipment-free high-intensity interval training (HEFHIIT) without dietary restrictions significantly improved systolic (SBP), diastolic blood pressure (DBP), and augmented resting post-prandial acyl ghrelin (AG). Notably, this training regimen did not alter body weight or other cardiometabolic risk markers. The subsequent study involving an 8-week HEFHIIT with/without the Mediterranean-style diet (MedDiet) demonstrated significant improvements in visceral adiposity and DBP, with weight loss and better body composition in the former group. HEFHIIT significantly increased fasting AG, while the addition of the MedDiet did not change fasting AG or des-acyl ghrelin (DAG) levels. Although no associations were identified between changes in ghrelin, weight or cardiometabolic risk markers, this thesis underscores the effectiveness of these easily implementable lifestyle behaviours in improving cardiometabolic markers without caloric restrictions. Notably, the absence of positive lifestyle behaviours over 8 weeks resulted in significant weight gain and reductions in insulin sensitivity. Improvements in abdominal adiposity and blood pressure are crucial determinants for maintaining long-term cardiometabolic health and post-menopausal women should be encouraged to adopt these lifestyle changes. Sustained efforts in maintaining these behaviours may yield clinically meaningful outcomes in the long term, beyond ghrelin mediation.

Declarations and Statements

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed: 

Date: 29 March 2024

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

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Date: 29 March 2024

The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

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Date: 29 March 2024

Publications and Public Engagement

Publications

Tan, A., Thomas, R. L., Campbell, M. D., Prior, S. L., Bracken, R. M., & Churm, R. (2023). Effects of exercise training on metabolic syndrome risk factors in post-menopausal women - A systematic review and meta-analysis of randomised controlled trials. Clinical nutrition (Edinburgh, Scotland), 42(3), 337–351.

Conference presentations

1. **Tan A.** Swansea University Sports and Exercise Science Postgraduate Conference, May 2023
2. **Tan A.** Diabetes UK Professional Conference, Award nominee Education and Self-management, April 2023
3. **Tan A.** Swansea University Sports and Exercise Science Postgraduate Conference, July 2022
4. **Tan A.** Welsh Endocrinology Diabetes Symposium, October 2021
5. **Tan A.** Global Challenges Postgraduate Research Symposium (Online), June 2021

Poster

1. **Tan A.** Diabetes UK Professional Conference, April 2024
2. **Tan A.** Diabetes UK Professional Conference, Award nominee Education and Self-management, April 2023.
3. **Tan A.** Diabetes UK Professional Conference, April 2023.
4. **Tan A.** Welsh Endocrinology Diabetes Symposium, May 2022
5. **Tan A.** Danish Diabetes Academy, Treatment of Diabetes, Obesity and Assessment of End Organ Damage (Kidney and Liver/Intestine) (Online), June 2021

Public Engagement

1. **Tan A.** Swansea Science Festival, October 2022. **Interactive Stand**
2. **Tan A.** Ageing Well Event (Information and Networking Event) at the LC Swansea, March 2022. **Interactive Stand**

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Abbreviations	Description
AACE	American Association of Clinical Endocrinology
AE	Aerobic Exericise
AEBSF	4-Benzenesulfonyl Fluoride Hydrochloride
AG	Acyl Ghrelin
AgRP	Agouti-Related Peptide
AHA/NHLBI	American Heart Association/National Heart, Lung And Blood Institute
ALSPAC	Avon Longitudinal Study of Parents And Children
AMH	Anti-Mullerian Hormone
AMPK	Amp-Activated Protein Kinase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
ARC	Arcuate Nucleus
AT	Aerobic Training
BAT	Brown Adipose Tissue
BHF	British Heart Foundation
BIA	Bio-Electrical Impedance Analysis
BMI	Body Mass Index
BP	Blood Pressure
CGM	Continuous Glucose Monitoring
CHD	Coronary Heart Disease
CMD	Cardiometabolic Disease
CMO	Chief Medical Officers
CNS	Central Nervous System
CONGA	Continuous Overall Net Glycaemic Action
CPET	Cardiopulmonary Exercise Test
CRF	Cardiorespiratory Fitness
CT	Combined Training
CV	Coefficient Variance
CVD	Cardiovascular Disease
DAG	Des-Acyl Ghrelin
DBP	Diastolic Blood Pressure
DEXA	Dual-Energy X-Ray Absorptiometry
DIO	Diet-Induced Obesity
E1	Oestrone
E2	Oestradiol
E3	Oestriol
ECG	Electrocardiogram
ECM	Extracellular Matrix
EDV	Endothelium-Dependent Vasodilation
EE	Energy Expenditure
EFHIIT	Equipment Free, High-Intensity Interval Training
EI	Energy Intake
ELISA	Enzyme-Linked Immunosorbent Assay
EMAS	European Menopause and Andropause Society
eNOS	Endothelial Nitric Oxide Synthase
ER	Endoplasmic Reticulum
ERMA	Estrogenic Regulation of Muscle Apoptosis
ERT	Oestrogen Replacement Therapy
FBG	Fasting Blood Glucose
FFA	Free Fatty Acids

FMD	Flow-Mediated Dilation
FSH	Follicular Stimulating Hormone
GH	Growth Hormone
GHRL	Pre-Proghrelin Gene
GHSR-1a	Growth Hormone Secretagogue Receptor 1a
GLP-1	Glucagon-Like Peptide 1
GnRH	Gonadotropin Releasing Hormone
GOAT	Ghrelin O-Acyltransferase
GV	Glycaemic Variability
HbA1c	Glycated Haemoglobin
HDL	High-Density Lipoprotein Cholesterol
HEFHIIT	Home-Based, Equipment Free, High-Intensity Interval Training
HIIE	High-Intensity Interval Exercise
HIIT	High-Intensity Interval Training
HOMA2	Homeostasis Model Assessment 2
HOMA2-%B	Beta Cell Function
HOMA2-%S	Insulin Sensitivity
HOMA2-IR	Insulin Resistance
HP	Hip Circumference
HPLC	High-Performance Liquid Chromatography
HR	Hazard Ratios
HR	Heart Rate
HRmax	Maximum Heart Rate
HRT	Hormone Replacement Therapy
IDF	International Diabetes Federation
IL-6	Interleukin-6
IPAQ	International Physical Activity Questionnaire
IQR	Interquartile Range
IR	Insulin Resistance
K_2EDTA	Dipotassium Ethylenediaminetetraacetic Acid
LDL	Low-Density Lipoprotein Cholesterol
LEAP2	Liver-Expressed Antimicrobial Peptide 2
LH	Luteinising Hormone
LPL	Lipoprotein Lipase
MD	Mean Difference
MedDiet	Mediterranean Diet
METs	Metabolic Equivalents (of Task)
MetS	Metabolic Syndrome
MICT	Moderate-Intensity Continuous Training
MONET	Montreal-Ottawa New Emerging Team
mRNA	Messenger RNA
MWHS	Massachusetts Women'S Health Study
NAFLD	Non-Alcoholic Fatty Liver Disease
NCEP:ATPIII	National Cholesterol Education Program Adult Treatment Panel Iii
NHS	National Health Service
NO	Nitric Oxide
NPY	Neuropeptide Y
OVX	Ovariectomised
Ox-LDL	Oxidised Low-Density Lipoprotein Cholesterol
PIS	Participant Information Sheet
POI	Premature Ovarian Insufficiency
PPARγ	Peroxisome Proliferator-Activated Receptor Γ

PUFAs	Polyunsaturated Fatty Acids
PVN	Paraventricular Nucleus
QC	Quality Control
RAAS	Renin-Angiotensin-Aldosterone System
RCT	Randomise Controlled Trial
RoB2	Cochrane Risk of Bias 2
ROI	Region of Interest
ROS	Reactive Oxygen Species
RPE	Rate of Perceived Exertion
RR	Relative Risk
RT	Resistance Training
SAT	Subcutaneous Adipose Tissue
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of the Mean
Ser3	N-Octanoylated Serine 3
SHBG	Sex Hormone Binding Globulin
SI	International System of Units
SNS	Sympathetic Nervous System
STRAW+10	Stages of Reproductive Aging Workshop +10
SWAN	Study of Women's Health Across The Nation
T2D	Type 2 Diabetes
tAUC	Total Area Under the Curve
TC	Total Cholesterol
TG	Total Ghrelin
TNF-α	Tumour Necrosis Factor Alpha
TRG	Triglycerides
UK	United Kingdom
UPR	Unfolded Protein Response
VAI	Visceral Adiposity Index
VAT	Visceral Adipose Tissue
VMS	Vasomotor Symptoms
VO_2	Aerobic Capacity
VO_{2max}	Maximum Aerobic Capacity
WAT	White Adipose Tissue

Chapter 1 – General Introduction

This chapter presents a brief outline of the research context and a problem statement, followed by the thesis aims and objectives.

1.1 Research Context

In this obesogenic society, there is a growing emphasis on therapeutic strategies to protect cardiometabolic health in aging. Obesity is strongly associated with cardiometabolic disease (CMD), significantly increasing both morbidity and mortality (Kim et al., 2021). The basis of weight gain results from imbalance within the energy balance equation, simply characterised as caloric intake exceeding energy expenditure (EE). Chronic overconsumption of calories with diminished physical activity creates a positive energy balance. Failure to address this imbalance can lead to obesity (defined by body mass index (BMI) ≥30 kg/m^2), excessive abdominal adiposity, and subsequent cardiometabolic risk.

Biological ageing introduces complexity to the relationship between physical activity and cardiometabolic health. Through gradual declines in physical capacity that can contribute to increased sedentary behaviour and physical inactivity, ageing affects the function of the body's regulatory systems that can increase risk of cardiometabolic dysfunction. These mechanisms include but are not limited to changes in body composition, altered energy production, increased inflammation and dysfunction of metabolic homeostasis (Bartke et al., 2021). To provide some context, the prevalence of cardiovascular disease (CVD) increases with age, with incidence risks of approximately 54%, 78% and 90% for age ranges 40 – 59, 60 – 79 and ≥80 years, respectively (Benjamin et al., 2019). Physical inactivity and sedentary behaviour can amplify this risk, with studies indicating a 5% increase in all-cause mortality for every 1-hour increment of sitting time beyond ≥7 hours/day (Chau et al., 2013).

A research demographic often overlooked are post-menopausal women. It should be noted that throughout this thesis, we refer to women as individuals biologically assigned as females at birth. The combination of ageing and hormonal changes poses substantial cardiometabolic risks in women. The lack of oestrogen during menopause predisposes women to an increased risk of metabolic abnormalities (Karvinen et al., 2019). Oestrogen deficiency alters body composition by decreasing lean body mass and promoting the redistribution of fat mass to the abdominal region, thereby increasing metabolic risk in this cohort (Lovejoy et al., 2008). Not only is oestrogen a cardiometabolic protector, but it has also shown to regulate caloric intake (Santollo & Daniels, 2019). During the follicular phase of the menstrual cycle where oestrogen levels are the highest, healthy pre-menopausal women have demonstrated to consume 14.5% less calories than in the luteal phase, where oestrogen levels are the lowest (Li et al., 1999). As a result, this can create a positive energy balance that can cause weight gain and further exacerbate metabolic dysregulation.

Many other hormones are understood to have a mediatory role in cardiometabolic risk. One such hormone, ghrelin, often referred to as the 'hunger hormone', is being explored as a therapeutic target for obesity-related comorbidities due to its influence on metabolic pathways (Churm et al.,

2017; Pulkkinen et al., 2010; Yuan et al., 2021). In addition to its role in appetite control and thus energy homeostasis, ghrelin influences glucose metabolism, insulin sensitivity and lipid metabolism (Sovetkina et al., 2020). Dysregulation of ghrelin is increasingly recognised for its association with cardiometabolic dysfunction, including obesity and metabolic syndrome (MetS) (Churm et al., 2017; Pulkkinen et al., 2010; Yuan et al., 2021). Individuals with high BMI exhibit lower fasting ghrelin levels compared to lean counterparts (Wang et al., 2022). Furthermore, low levels of ghrelin associated with MetS have been found to decrease as metabolic abnormalities intensify (Ukkola, 2009). Understanding ghrelin's relationship to metabolic homeostasis is paramount for addressing the escalating CMD risk. Utilising lifestyle interventions, the specific mechanisms through which ghrelin interacts with cardiometabolic risk factors as well as the modulation of its activity represent promising areas for research.

1.2 Problem Statement

Though it is known that both regular physical activity and/or healthy dietary choices can confer improvements in cardiometabolic risk reduction (Sovetkina et al., 2020), there is limited research exploring the potential positive effects of lifestyle modification in post-menopausal women. Furthermore, it is unclear if ghrelin plays a role in any adaptations. In healthy post-menopausal women, studies have shown reduced circulating ghrelin levels to be associated with multiple changes in CVD risk factors (Wildman et al., 2008), while others report no such associations (Iwamoto et al., 2005; Karakus et al., 2012; Purnell et al., 2003). From the review of the extensive literature, it is clear that this area remains under researched in post-menopausal women, particularly in the mediation of the ghrelin axis and its influence in cardiometabolic risk markers with physical activity and diet.

1.3 Thesis Aims and Objectives

The overall aim of this thesis is to explore the efficacy of lifestyle interventions with exercise and diet to alleviate cardiometabolic risk factors, while exploring the role of ghrelin mediation in sedentary and overweight post-menopausal women. This knowledge may contribute to the better understanding of the role of ghrelin in aiding mitigation of CMD risk in post-menopausal women.

This thesis consists of three main chapters:

1) Chapter 4 investigates the impact of different exercise modalities on cardiometabolic risk factors in post-menopausal women through systematic review and meta-analysis.
2) Chapter 5 explores the impact of high-intensity interval training (HIIT) on cardiometabolic risk factors and ghrelin levels acutely (laboratory-based single dose) and over an 8-week home-based HIIT intervention.
3) Chapter 6 establishes the cardiometabolic risk factors and ghrelin response as well as explores their relationship to 8-week HIIT with or without the Mediterranean diet.

Chapter 2 – Literature Review

This literature review chapter provides the background and rationale for the thesis, encompassing an overview of CMD, lifestyle-related modifiable and non-modifiable cardiometabolic risk factors, the menopause, and the gut hormone ghrelin.

2.1 Cardiometabolic Disease

2.1.1 Definition and Prevalence of Cardiometabolic Disease

The prevalence of CMD including MetS, type 2 diabetes (T2D) and CVD is increasing rapidly and are the leading causes of morbidity and mortality in both sexes worldwide (Benjamin et al., 2019). According to a 2018 biobank data analysis of middle-aged adults (40 to 69 years of age) in the United Kingdom (UK), it was reported that nearly a-fifth (19%) of the UK population have multimorbidity, of which a combination of CVD and diabetes was the most prevalent (Zemedikun et al., 2018). Complementary to this, a more recent finding from 2022 found that risk of total mortality was positively correlated with the number of CMDs, evident from hazard ratios (HR) for mortality of 1.49, 2.17 and 3.75 for one, two and three CMD conditions, respectively (Xu et al., 2022).

2.1.2 Cardiovascular Disease

CVD is a group of disorders and events associated with the heart and blood vessels, including cerebrovascular disease (stroke, ischemia), coronary heart disease (CHD; myocardial infarction, heart failure) and peripheral vascular disease (aortic atherosclerosis) (Benjamin et al., 2018). It is the leading cause of mortality and morbidity worldwide, with the World Health Organisation (WHO) reporting an estimated of 17.9 million CVD-related deaths in 2019. This represents 32% of all global deaths, of which 80% are due to stroke and CHD (World Health Organisation, 2019). In 2016, the British Heart Foundation (BHF) approximated 152,000 individuals in the UK to have died from CVD, with 11% from CHD and 6% from stroke. Furthermore, the BHF reported that 2.3 million people are currently living with CHD (British Heart Foundation, 2018).

Atherosclerosis is the main underlying cause of CVD-related mortality worldwide. It is a chronic inflammatory condition characterised by the thickening and hardening of the arterial walls from the formation of atherosclerotic lesions, consequently decreasing or impeding blood flow within the blood vessels (Libby et al., 2011). Atherosclerosis is initiated by multiple factors that trigger the formation of *the fatty streak*, a hallmark of atherosclerotic plaque development, including dyslipidaemia, inflammation, oxidation and endothelial dysfunction (Davies et al., 1988). The initial stage begins with endothelial dysfunction, where factors such as hypercholesterolaemia, smoking and hypertension increase endothelial permeability, thereby allowing migration of low-density lipoprotein (LDL) into the vascular wall (Bergheanu et al., 2017). Trapped LDL within the vascular wall undergo lipid peroxidation and become oxidised LDL (ox-LDL) due to local secretion of reactive oxygen species (ROS). Consequently, the endothelial cells express adhesion molecules and produce several inflammatory mediators, leading to the adhesion of circulating monocytes to the lesion site. Upon migration into the arterial wall, the monocytes differentiate into macrophages. The hallmark of fatty streak development forms when these macrophages and

proliferated smooth muscle cells engulf the ox-LDL particles, forming foam cells. Overtime, the lesion continues to expand as more LDL particles are oxidised, precipitating further macrophage recruitment and the inflammation is further exacerbated, forming the atherosclerotic plaque (Bergheanu et al., 2017). Genetics and lifestyle factors heavily influence the development and severity of atherosclerosis, and resultant progression of multimorbidity. A pivotal risk factor, obesity, can result in chronic inflammation, contributing to the progression of atherosclerosis (Henning, 2021). It is therefore important to address modifiable risk factors such as lifestyle behaviours to prevent and reduce progression of obesity.

2.1.3 Type 2 Diabetes

T2D is a form of diabetes mellitus characterised by hyperglycaemia, resulting from impaired insulin secretion by the pancreatic β-cells and/or the inability of insulin-sensitive tissues to respond to insulin (International Diabetes Federation, 2021). The global incidence of T2D has increased substantially over the past thirty years, where trends of T2D have increased significantly by nearly thirteen-fold from 30 million in 1985 to 382 million in 2014. Latest estimates from the International Diabetes Federation (IDF) approximated 536.6 million people (10.5%) with T2D in 2021, and this number has been forecasted to rise to 783.2 million people (12.2%) by 2045 (Sun et al., 2022).

In a state of chronic exposure to hyperglycaemia, T2D is associated with damage and dysfunction of the heart, eyes, vasculature, nerves and the kidneys (International Diabetes Federation, 2021). Consequently, microvascular (nephropathy, neuropathy and retinopathy) and macrovascular (cardiovascular, cerebrovascular and peripheral vascular) complications develop, leading to a two to four-fold increased risk of CVD (Harding et al., 2019). It has been estimated that CVD affects 32.2% of individuals with T2D (Einarson et al., 2018), and can reduce life expectancy as high as 10 years (International Diabetes Federation, 2021). CVD is a major cause of disability and mortality amongst individuals with T2D, with the main contributors being stroke and coronary artery disease.

The main drivers of T2D manifestations are ageing, genetics and an obesogenic environment promoting unhealthy diet and sedentarism (Zhou et al., 2016). Approximately 80 – 90% of patients are overweight or obese, and studies report that having both conditions can increase mortality risk by seven-fold (Leitner et al., 2017; Oldridge et al., 2001). In a healthy individual, the tightly regulated feedback loop of insulin secretion and insulin action work in tandem to meet metabolic demand, thus maintaining a euglycemic level in the blood. In T2D, pancreatic β-cell dysfunction impairs insulin secretion, limiting the body's capacity to maintain physiological glucose levels, causing hyperglycaemia. Additionally, the pathophysiology of T2D can also result from peripheral insulin resistance (IR). Obesity-associated metabolic dysfunctions including excessive adiposity, adipokine dysregulation, increased inflammation and free fatty acids (FFA) secretion promote IR of insulin sensitive organs such as the liver, muscle and adipose tissue (Henson et al., 2023). The pathophysiology of these mechanisms is further discussed from section 2.2.2 onwards.

2.1.4 Metabolic Syndrome

MetS is defined by a cluster of risk factors of metabolic origin that is linked to the predisposition of CMD, including CVD, T2D, atherosclerosis and stroke (Tune et al., 2017). These collective risk factors include increased waist circumference (WC), elevated blood pressure (BP), fasting blood glucose (FBG) and triglyceride (TRG) levels and decreased high-density lipoprotein cholesterol (HDL) levels (Grundy et al., 2005). Over the past several decades, international health organisations including the WHO (Alberti et al., 1997), the American Association of Clinical Endocrinology (AACE) (Bloomgarden, 2003), The National Cholesterol Education Program Adult Treatment Panel III (NCEP:ATPIII) (Expert Panel on Detection, 2001), American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) (Grundy et al., 2005) and the IDF (Alberti et al., 2005) have issued varying definitions of MetS. For example, the AHA/NHLBI, NCEP:ATPIII and the IDF used WC as the surrogate marker for abdominal obesity, whereas IR was the main focus for MetS by AACE and WHO. To date, there is a consensus of the definition of MetS. Due to the complexity of the condition, it was harmonised that three out of the five risk factors would qualify the diagnosis of MetS (Alberti et al., 2009) (Table 2.1).

Table 2.1. Consensus definition of Metabolic Syndrome Criteria by IDF and AHA/NHLBI (2009) (Alberti et al., 2009)**.** *International System of Units (SI) are reported and converted from mg/dL to mmol/L.

Component (any of the three)	Clinical Cut-off Values
Waist Circumference	≥102 cm in men ≥88 cm in women
Triglycerides*	≥1.69 mmol/L
HDL Cholesterol*	<1.03 mmol/L in men <1.29 mmol/L in women
Fasting Blood Glucose*	≥5.6 mmol/L
Blood Pressure (BP)	≥130 mmHg Systolic BP ≥85 mmHg Diastolic BP

In individuals with MetS, the risk of CVD events such as stroke and myocardial infarction is twice as high compared to those without MetS (Mottillo et al., 2010). Additionally, the risk of cardiovascular events increases with increasing number of MetS components (Guembe et al., 2020). The risk of T2D with MetS components has also been established. The prospective Copenhagen General Population Study found an increased 5% risk of T2D for every centimetre increase in WC. Furthermore, they also found an increased risk of 32% of T2D for every 1 mmol/L increment of FBG (Marott et al., 2016). In a longitudinal study of 10.8 million South Koreans, Lee *et al.* reported a 46% decreased risk of T2D with improvement in FBG, and an 8% decrease with

reduced abdominal obesity (Lee et al., 2020). As MetS precedes development of CMD, it is paramount to manage risk factors of MetS to mitigate disease progression.

2.2 Abdominal Obesity: Pathophysiology of Cardiometabolic Disease

The pathophysiology of CMD is well documented. Obesity, particularly abdominal obesity, is associated with MetS and is driven by factors including IR, dyslipidaemia, inflammation, and adverse cardiovascular effects that contribute to the manifestation of CMD. Abdominal obesity is defined by excess deposits of adipose tissue in the abdominal region and has adverse implications on cardiometabolic dysfunction (Valenzuela et al., 2023) (see sections below from 2.2.2). Globally, the prevalence of overweight and obesity has escalated alarmingly and have reached pandemic proportions in the past decade, where rates of obesity have nearly tripled since 1975 (WHO, 2020). The WHO defines overweight and obesity as 'abnormal or excessive fat accumulation that presents a risk to health'. Specifically, the National Health Service (NHS) defines a BMI of 25 – 29.9 kg/m^2 as overweight, and a BMI of ≥ 30 kg/m^2 as obese. The World Obesity Atlas 2023 reports the estimated prevalence of adults with obesity in the UK to be 33%, of which this is expected to increase to 45% in 2035 (Lobstein et al., 2023). In Wales alone, The National Survey indicated that 62% of adults were overweight or obese in 2020, of which 25% were obese (The Welsh Government, 2020). Obesity is a multifaceted chronic disease caused by the complicated interplay of genetic, behavioural, socioeconomic, metabolic and environmental factors, leading to increased risk in morbidity and mortality. The risk of developing CMD multimorbidity with obesity is almost five-fold, and almost fifteen-fold for obesity classes two and three (Kivimäki et al., 2017). Furthermore, a recent umbrella review and meta-analysis of observational and mendelian randomisation studies of 30 million participants comprehensively described the increased risk of incident of CVD and mortality with increased BMI and adiposity (Kim et al., 2021). Kim *et al.* found a 10% relative risk (RR) for haemorrhagic stroke and 49% RR for hypertension for every 5 kg/m^2 BMI increment (Kim et al., 2021). The consequential health risks associated with obesity is well documented in a plethora of literature, not limited to MetS (Engin, 2017), CVD (Dwivedi et al., 2020), T2D (Abdullah et al., 2010), non-alcoholic fatty liver diseases (Polyzos et al., 2019), and cancer (Ackerman et al., 2017). The pathophysiological mechanism of carrying excess adiposity in the development of MetS, CVD and T2D is described in Figure 2.1.

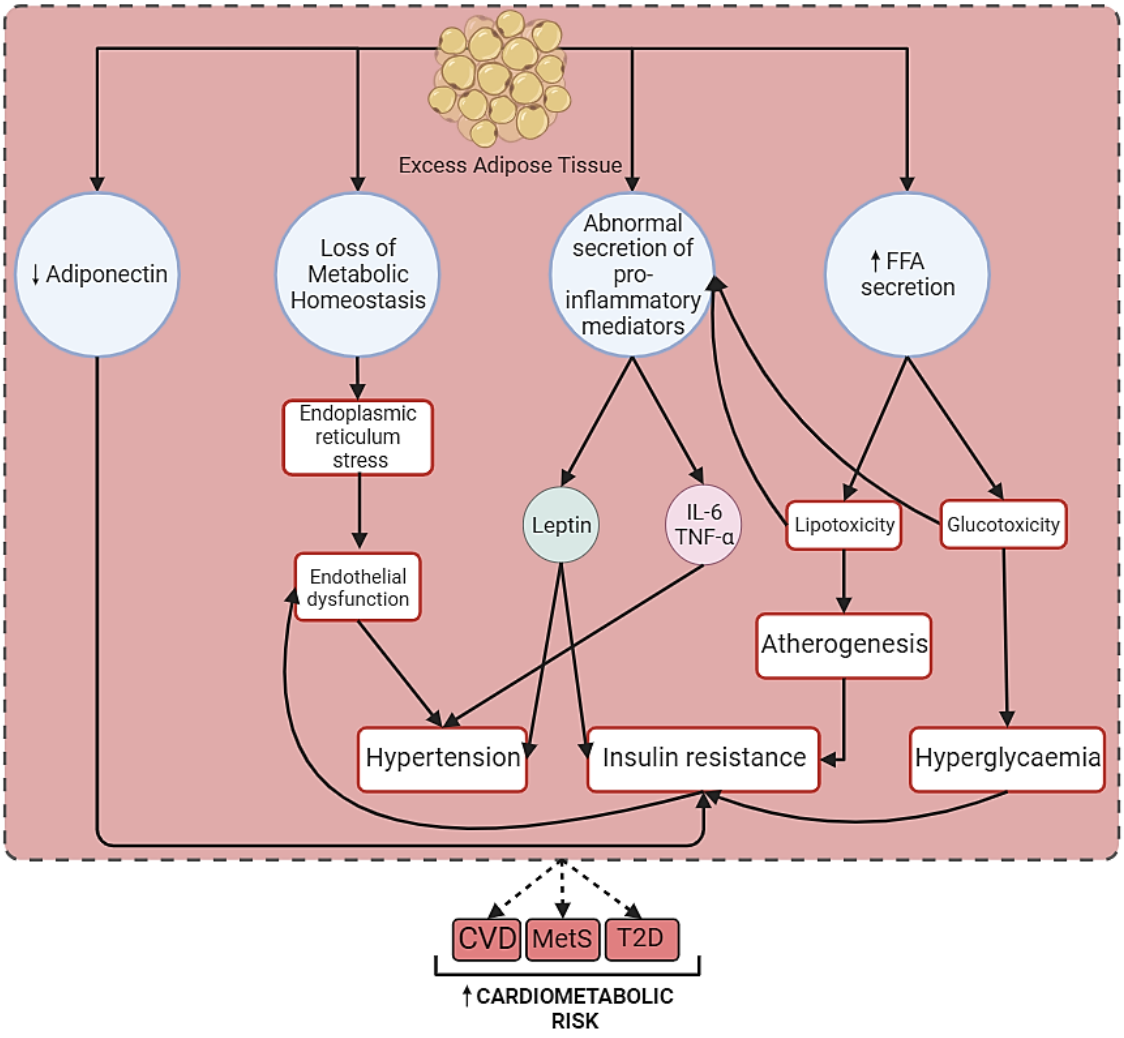

Figure 2.1 Summary of the main mechanisms associated with the pathophysiology of excess-adiposity related cardiometabolic risk. FFA: free fatty acid; IL-6: interleukin-6; TNF-α: tumour necrosis factor alpha; CVD: cardiovascular disease; MetS: metabolic syndrome; T2D: type 2 diabetes. ***(Own image).***

2.2.1 Adipose Tissue

To understand the disease pathophysiology of carrying excess adiposity, it is important to first understand the function of adipose tissue. Adipose tissue is an important endocrine organ and an essential organ for energy homeostasis regulation. It is compartmentalised in the human body in two basic categories: white adipose tissue (WAT) and brown adipose tissue (BAT). Briefly, BAT serves as a thermogenic organ for heat production (Cannon & Nedergaard, 2004). WAT is the most abundant form of adiposity, approximating 5 – 50% of body weight and is stored in two distinct depots: subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) (Ibrahim, 2010). In this thesis, reference to adipose tissue or adiposity pertains to WAT.

2.2.1.1 Subcutaneous Adipose Tissue

SAT comprises of approximately 80% of total body fat mass in lean, healthy individuals, and is distributed throughout the body, primarily localised to the upper and lower body depots. In addition to its role in providing insulation and physical protection, SAT is the largest and the preferential depot for excess fat storage in the form of neutral lipid triacylglycerol which can be readily mobilised when needed (Ibrahim, 2010). However, when its storage capacity is exceeded, either by inadequate generation of new adipocytes (limited hyperplasia) or the incapacity to further enlarge existing adipocytes (limited hypertrophy) fat starts to accumulate in regions beyond SAT (Kwok et al., 2016).

2.2.1.2 Visceral Adipose Tissue

In contrast to SAT, VAT is located mainly in the abdominal region, accounting for 5 – 20% of total body fat mass (Ibrahim, 2010). Lean, healthy individuals do not have large amounts of visceral fat. VAT is highly metabolically active and continuously releases FFA into the portal circulation. While FFA is essential for energy homeostasis, the pathogenesis of excess VAT begins when its lipid storage capacity is exceeded. This results in lipid spillage into the ancillary organs like the liver, thus adversely affecting the organ's metabolic function, such as hepatic IR (Hammarstedt et al., 2018). Accumulation of excess fat to accommodate energy reserves in other organs or tissues is known as ectopic fat. In women with morbid obesity, VAT adipocyte hypertrophy was associated with IR and other metabolic dysfunctions such as hypertension (Ledoux et al., 2010). Consequently, visceral fat contributes to multiple aspects of MetS, including systemic inflammation, hyperinsulinemia, dyslipidaemia and hypertension (Chait & den Hartigh, 2020). This is further expanded below under 'Adipose Tissue Dysfunction', section 2.2.2.

2.2.1.3 Hypertrophy versus Hyperplasia

Adipose expansion occurs through increased adipocyte numbers (hyperplasia) or swelling of individual adipocytes (hypertrophy). WAT depots contain preadipocytes, which are the precursor of mature adipocytes, accounting for 15 – 50% of cells in fat tissue (Tchkonia et al., 2013). Adipogenesis is the process where preadipocytes develop to mature adipocytes, regulated by

various transcription factors and hormones. Two subtypes of preadipocytes exist with characteristics that oppose each other, with one type (hyperplasic) demonstrating greater proliferation, replication, adipogenesis and resistance to TNF-α apoptosis than the other (hypertrophic) (Tchkonia et al., 2005). Pre-adipocytes from SAT exhibit characteristics akin to the former subtype and is associated with hyperplasia, facilitating vascularisation and promotes anti-inflammation thus maintaining tissue health. In contrast, subtypes from the latter are present in VAT and is associated with hypertrophy, characterised by attenuated vascularisation and increased inflammation, contributing to the pathology of CMD (Steiner & Berry, 2022). Therefore, SAT expansion, in part, is a process that is metabolically healthy, even in severe obesity (Laforest et al., 2015). For example, gastric by-pass patients with morbid obesity (average BMI 41 kg/m^2) presented with favourable cardiometabolic profile that was associated with a higher SAT cell number, evident by higher insulin sensitivity, HDL levels and lower TRG levels (Rydén et al., 2014). Additionally, SAT hyperplasia serves as a protective role against dyslipidaemia, dysglycaemia and insulin abnormalities in women with morbid obesity (Hoffstedt et al., 2010).

2.2.2 Adipose Tissue Dysfunction

Adipose tissue is a dynamic organ capable of responding to its environmental inputs. In response to a chronic positive energy balance, adipose tissue undergo restructuring by altering the number and size of mature adipocytes. Simultaneously, precursor cells are recruited and committed to the adipocyte lineage when adipocyte size reaches critical threshold. During this process, the hypertrophic adipocytes release paracrine factors, including hormones and cytokines, facilitating the recruitment of preadipocytes and promote adipogenesis (Pellegrinelli et al., 2016). Adipose tissue hyperplasia is regarded as a ‘recovery mechanism’ to overnutrition (Spalding et al., 2008) by attempting to repair metabolic alterations when adipocytes become lipid-overloaded and IR (Blüher, 2016). To accommodate massive adipose tissue expansion, the extracellular matrix (ECM), a complex structure composed of different proteins that provides a scaffold for cells involved in modulating biological processes, undergo remodelling and reorganisation that is paramount to accommodate sufficient space for hypertrophy and hyperplasia (Ruiz-Ojeda et al., 2019). Collectively, these processes constitute the term ‘adipose tissue remodelling’ (Choe et al., 2016).

In obesity, adipose tissue dysfunction refers to the unhealthy expansion of adipose tissue, particularly an increase in VAT mass (Santillana et al., 2023). Adipose tissue dysfunction is associated with numerous deleterious effects, including hypoxia and lipotoxicity, contributing to metabolic dysfunction. Furthermore, disruptions in adipose tissue remodelling can also lead to dysregulated secretion of adipocytokines by adipose tissue, resulting in local and systemic inflammation, cascading further into other metabolic disturbances (Lipke et al., 2022).

2.2.2.1 Hypoxia

As adipose tissue expands in obesity, the formation of new vessels is crucial which promotes adipocyte differentiation. During healthy adipose tissue expansion, there is an equilibrium

between adipocyte hypertrophy and hyperplasia, with adequate vascularisation and functional ECM remodelling (Santillana et al., 2023). Adipose tissue hypoxia occurs as a consequence of adipocyte hypertrophy and dysfunctional vasculature, resulting in hypoxic regions inside the tissue (Ruiz-Ojeda et al., 2019). In response, adipocytes activate hypoxia-inducible factor 1-alpha, an important signalling molecule for hypoxia that induces the inflammatory response and ECM components (Santillana et al., 2023). Since collagen compromises the main component of ECM, excessive expansion of ECM increases collagen accumulation, thus decreasing ECM flexibility and can hinder adipocyte expansion. Consequently, local fibrosis develops, triggering cell necrosis and inflammation within the adipose tissue. Furthermore, this causes the adipose tissue to exceed its capacity for fat storage, resulting in lipotoxicity (Ruiz-Ojeda et al., 2019; Santillana et al., 2023).

2.2.2.2 Lipotoxicity

To accommodate for excess lipid storage, deleterious ectopic lipid accumulation in other cell types other than adipocytes is known as lipotoxicity. These include cells of organs such as the liver (hepatocytes), pancreas (β-cells) and skeletal muscles (myocytes). As previously mentioned, hypertrophic adipose tissues in VAT constantly release FFA into circulation (Longo et al., 2019). When FFA accumulation exceeds FFA clearance, these non-adipocyte cells have limited lipid storage and the lipotoxic milieu impairs cellular homeostasis and disrupts tissue function. Mechanisms of lipotoxicity includes oxidative stress, endoplasmic reticulum (ER) stress, inflammation and IR (Lipke et al., 2022).

Oxidative stress

Reactive oxidative species (ROS) are byproducts of cellular metabolism and play a supporting role in numerous diseases (Bardaweel et al., 2018). The imbalance between ROS and anti-oxidative defence mechanisms induced from adipocyte hyperplasia and hypertrophy results in free radicals within adipose tissue, leading to oxidative stress (Keaney et al., 2003). Oxidative stress can irreversibly damage or destroy cellular components such as proteins, lipids, carbohydrates and DNA, further exacerbating the inflammatory response. Excess FFA content in non-adipose tissue cells such as hepatocytes render mitochondria dysfunctional, leading to increased cytosolic production of ROS. The resultant accumulating oxidative damage can further exacerbate mitochondrial dysfunction and further promote ROS production, thereby initiating a vicious cycle (Lipke et al., 2022).

Endoplasmic reticulum stress

In the ER, proteins fold into their native conformation and undergo numerous post-translational modifications. Mildly stressful conditions in the ER such as disruptions in these processes lead to buildup of unfolded and aggregated proteins, prompting the activation of the unfolded protein response (UPR), an adaptive mechanism that restores normal ER homeostasis. However, in the presence of chronic FFA exposure, FFAs can induce ER stress by activating UPR. Unresolved

FFA-mediated ER stress exacerbates inflammation, IR and apoptotic pathways that contribute to systemic metabolic disturbances (Lipke et al., 2022).

2.2.2.3 Inflammation and Insulin Resistance

Excess VAT and lipotoxicity is associated with the secretion of numerous pro-inflammatory molecules into the circulatory system, resulting in chronic, low-grade inflammation that leads to IR. The inflammation associated with excess VAT is caused by the loss of metabolic homeostasis in adipocytes, stemming from the excess influx of circulating nutrients. The preliminary response to increased energy storage is initially accommodated by adipocyte enlargement and hyperplasia. However, eventual lipid overload of hypertrophic adipocytes causes ER and oxidative stress, resulting in increased production of pro-inflammatory markers to serve as an adaptive protective response (Keaney et al., 2003; Lemmer et al., 2021). However, with chronic overnutrition, these protective actions are insufficient, leading to the death of adipocytes and senescence of several tissue cell type, including hepatocytes. The consequential structural tissue damage and loss of metabolic homeostasis is accompanied by the activation of the immune system, involving the expression or release of immunostimulatory cell components and immune cell types (Kolb, 2022).

Hypertrophic VAT and lipotoxic non-adipose organs secrete pro-inflammatory adipokines including leptin, interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) as well as decreased secretion in anti-inflammatory adipokines such as adiponectin (Kolb, 2022).

Leptin

Leptin regulates energy balance and regulates glucose-insulin metabolism via exerting its effects on the hypothalamus. Circulating leptin is proportional to fat mass, where an increase in leptin concentrations reduces appetite and body weight. Leptin increases fatty acid oxidation and fatty acid update, reduces TRG formation, and promotes thermogenesis (Pereira et al., 2021). However, in obesity where hyperleptinaemia is observed (Pereira et al., 2021), the efficacy of leptin action is decreased, a consequence of inadequate leptin-mediated response known as leptin resistance (Liu et al., 2018). The attenuated leptin-mediated action in the brain causes excess TRG to accumulate in non-adipose and adipose tissue, subsequently impairing insulin sensitivity and secretion (Steinberg et al., 2002). Thus, leptin resistance is also associated with IR (Shih et al., 2022). As a pro-inflammatory cytokine, leptin has several adverse multisystemic roles that contribute to further inflammation and risk of CMD. For example, leptin secretion from VAT induces endothelial cell dysfunction and inflammation, leading to vasoconstriction and promotion of atherosclerotic plaque formation (Vasamsetti et al., 2023).

Adiponectin

In contrast to leptin, circulating adiponectin is inversely proportional to total fat mass, with lower levels reported individuals with obesity compared to lean individuals (Straub & Scherer, 2019). Adiponectin possess cardioprotective effects including improving muscle and hepatic insulin sensitivity, fatty acid oxidation, as well as decreasing hepatic gluconeogenesis and lipogenesis

(Straub & Scherer, 2019). As such, low adiponectin levels have been associated with IR in individuals with obesity and T2D, and patients with CHD (Berg et al., 2002).

Interleukin-6

Compared to SAT, VAT secretes 2 to 3-fold times more IL-6, contributing to the pathogenesis of hepatic IR (Fried et al., 1998). Chronic exposure to pro-inflammatory mediators activates cytokine signalling proteins, leading to the inhibition of insulin signalling receptor activation in the β-cells of the pancreatic islets. For example, IL-6 activates the suppressor of cytokine signalling (Kawazoe et al., 2001), which serves to inhibit components of the cytokine signalling cascade, thereby blocking the cytokine-mediated transcriptional factor that activates the insulin receptors (Krebs & Hilton, 2000).

Tumour necrosis factor alpha

Similarly, secretion of TNF-α from adipocytes triggers a spectrum of signalling cascade upon binding with its receptor, leading to the activation of various transcriptional pathways associated with the impairment of insulin action through the phosphorylation of serine on insulin receptor substrate-1 (Aguirre et al., 2000). Furthermore, IL-6 suppresses lipoprotein lipase (LPL) which is responsible for the degradation of TRG, resulting in elevated TRG levels (Kern et al., 2001). As such, both IL-6 and TNF-α circulating levels correlate positively with BMI, adiposity, insulin levels and IR (Zahorska-Markiewicz et al., 2000).

2.2.3 Dyslipidaemia

Fat accumulation is determined by the subtle balance between lipolysis (fat breakdown) and lipogenesis (fat synthesis). The imbalance of these variables lead to excess adipose accumulation, resulting in the release of excess FFA and a cascade of processes involving the disruption of metabolic homeostasis (Saponaro et al., 2015). Dysregulation in FFA metabolism is associated with the pathogenesis of dyslipidaemia and hyperglycaemia, contributing to prevalence of CMD (Kirk & Klein, 2009). VAT is highly metabolically active with a higher capacity of lipolysis, causing excessive FFA to be continuously released into circulation (Frayn et al., 2003). Thus, increased abdominal adiposity is strongly associated with adverse dysregulated lipid metabolism that mirrors an atherogenic dyslipidaemic profile, characterised by reduced HDL, increased LDL and hypertriglyceridemia (Ko & Jung, 2021). As previously mentioned, lipotoxicity occurs when the deleterious effects of high concentrations of lipids and its derivatives accumulate in non-adipose tissues, particularly the liver. The liver is a major target in the highly concentrated environment of inflammatory markers and FFA. In the cytoplasm of hepatocytes, FFA-derived TRG accumulate in the form of lipid droplets of which various intermediate lipid moieties have been shown to promote lipotoxicity and hepatic IR (Perry et al., 2014). Furthermore, the secretion of FFAs can inhibit insulin-stimulated glucose uptake, glucose oxidation and glucose synthesis, resulting in glucotoxicity (Boden, 2001).

2.2.4 Hypertension

Hypertension, defined by the NHS as BP of ≥140/90 mmHg, is complex and involves many factors which leads to vascular damage. Obesity, particular abdominal obesity, is strongly associated with the pathogenesis of hypertension (El Meouchy et al., 2022). The Framingham study demonstrated that nearly 50% of middle-aged adults (50 – 59 years) with obesity were hypertensive (Kannel et al., 1967). The investigation of body adipose tissue distribution and prevalence of hypertension is well established. In a recent study of 436 patients with obesity, Tałałaj *et al.* found the prevalence of hypertension to be 64.5% in those with BMI <40 kg/m^2, and 78.7% in those with BMI ≥50 kg/m^2 (Tałałaj et al., 2023). Additionally, visceral adiposity index (VAI), a surrogate marker of visceral adipose distribution based on anthropometric parameters (BMI and WC) and biochemical parameters (TRG and HDL), have been found to be positively associated with SBP (Leite et al., 2021).

The excessive adipokines secreted by adipose tissue contribute to endothelial dysfunction. Molecules including angiotensinogen, aldosterone, angiotensin II and renin, formulate part of the renin-angiotensin-aldosterone system (RAAS), a core regulating system for BP (Fountain et al., 2023). These peptides, in addition to the secretion of IL-6 and TNF-α, are secreted by VAT that collectively elevate arterial pressure via vasoconstriction and sodium retention, resulting in hypertension (Fountain et al., 2023). The pathogenesis of hypertension can also be manifested from IR. Evidence show that an IR state impairs the insulin-stimulated nitric oxide (NO) pathway, activating the mitogen-activated protein kinase pathway and contributing to vasoconstriction, thus increasing BP (Zhou et al., 2014). Antonio-Villa e*t al.* show that VAT is a mediator of the effect of IR on BP, where individuals with both conditions presented with 69.1% higher risk of incident hypertension (Antonio-Villa et al., 2021).

2.3 Lifestyle-related modifiable and non-modifiable risk factors on cardiometabolic risk

Contributions to increased risk of CMD can be underpinned to lifestyle-related modifiable and non-modifiable risk factors. Lifestyle modifications associated with elevated CMD risk include but are not restricted to: excessive alcohol consumption, smoking and tobacco use, sedentary behaviour and physical inactivity as well as poor dietary habits. In addition, non-modifiable risk factors include genetics and family history, age and sex. For the purpose of the thesis, elaboration of lifestyle-related modifiable risk factors will be limited to sedentary behaviour, physical inactivity and diet, and limited to age and sex for non-modifiable risk factors.

2.3.1 Lifestyle Modifications on Reducing Cardiometabolic Risk

In an obesogenic society, lifestyle factors largely contribute to the dramatic rise in prevalence for CMD in the past few decades. With 90% of patients with T2D with overweight or obesity (Grant et al., 2021), abdominal obesity and carrying excess weight are key modifiable factors in the development of this disease.

2.3.1.1 Physical Inactivity and Sedentary Behaviour

Public health guidelines recommend regular physical activity of moderate intensity of at least 150 minutes per week, or 75 minutes of vigorous physical activity a week to maintain health or prevent major chronic diseases (Davies et al., 2019). In a dose-response manner, substantial decrease in risk of mortality was reported to be associated with higher levels of time spent performing physical activity, combined with intensity, frequency and duration (Ekelund et al., 2019). Additionally, maximal risk reductions for mortality were observed with 24 minutes/day of moderate-vigorous physical activity, as well as 375 minutes/day of light-intensity physical activity (Ekelund et al., 2019). Monitoring step count acquired daily through accelerometers and fitness device tracking have become a feasible method of measuring physical activity. A meta-analysis also revealed an association between increased step-count and lower risk all-cause mortality that was varied by age (Paluch et al., 2022). For example, a step count of 7500 steps/day in older women (≥62 years) (Lee et al., 2019) and step counts of 8000 – 12000 in middle-aged adults (≥40 year) was associated with lower mortality rates (Saint-Maurice et al., 2020).

In 2022, the WHO reported that 27.5% of the global adult's population do not meet recommended physical activity levels (World Health Organization, 2022), a contributing factor to premature mortality of 7.2% of all-cause and 7.6% of CVD deaths (Katzmarzyk et al., 2022). In addition to physical inactivity, sedentary behaviour also poses a major problem. A concept distinct from physical inactivity, sedentary behaviour is defined as "any waking behaviour characterized by an energy expenditure (EE) ≤1.5 metabolic equivalents (of task) (METs), while in a sitting, reclining or lying posture" (Tremblay & Therrien, 2006). Moreover, individuals that do adhere to the recommended physical activity guidelines may still engage in sedentary behaviour, thereby not fully mitigating associated health risks (Rantalainen et al., 2018).

Aside from the chronic positive energy balance that can incur from long-term physical inactivity and sedentary behaviour, the exact physiological mechanisms behind the adverse effects on the human body are unknown. Several proposed hypotheses on their impacts have been suggested including cardiovascular burden through impaired lower limb vascular function (Paterson et al., 2020) and increased peripheral BP (Paterson et al., 2022). Additionally, physical inactivity also contributes to reduced levels of LPL activity in plasma and skeletal muscles (Miyashita et al., 2010), further contributing to a metabolically unfavourable lipid profile. Glucose metabolism is also impaired due to a downregulation of skeletal muscle contraction-mediated glucose update (Higgins et al., 2022). Repeated exposure to acute bouts of sedentary behaviour and physical inactivity result in dysfunctional metabolic pathways, contributing to increase systemic inflammation and further exacerbating cardiometabolic dysfunction.

Higher durations of sedentary time are positively correlated with increased risk of mortality, of which this risk increased at durations >9.5 hours each day (Ekelund et al., 2019). Even with moderate-vigorous physical activity considered, it was observed that for every 1-hour increment in sitting time from ≥7 hours/day, all-cause mortality increased by 5% (Chau et al., 2013). Regular

interruptions of prolonged sitting with physical activity have been found to offset the acute detrimental effects of prolonged sedentary behaviour by maintaining muscle pump and blood flow (Paterson et al., 2020, 2022), further highlighting the importance of maintaining a physically active lifestyle.

2.3.1.2 Poor Dietary Habits

Prolonged excessive caloric consumption can promote a chronic state of positive energy balance, largely contributing to weight gain and obesity. Furthermore, poor dietary consumption habits also contribute to risk factors associated with early mortality and disability in European countries (Gakidou et al., 2017). A systematic evaluation of dietary consumption patterns across 195 countries reported that non-optimal intake of whole grains, fruits and sodium accounted for 50% of deaths and 60% of disabilities related to diet (Afshin et al., 2019). Although consumption of sodium, saturated fats and sugars are the main focus in combating unhealthy diet as reported by the WHO (World Health Organisation, 2013), the leading dietary risk factors of low consumptions in whole grains, fruits, nuts and seeds, vegetables, omega-3 fatty acids, combined with high sodium, accounts for more than 2% of deaths globally (Afshin et al., 2019). Previous studies show that diets high in these components and low in sugars, red meat and processed food are associated with lower mortality and have cardiometabolic benefits (Afshin et al., 2019; Levy & Tedstone, 2017). Foods high in saturated fats promote unfavourable lipid profiles by increasing TRG and LDL concentrations. Furthermore, red meat and processed foods promote low-grade inflammation, a key contributor in the pathogenesis of non-communicable diseases including T2D and CVD (Wang et al., 2022). Conversely, increased consumption of dietary fibre can support regulation of glucose homeostasis, lower TC levels and possess anti-oxidative properties, all of which contribute to decreased risk of CMD (Threapleton et al., 2013).

As previously mentioned, WC is a surrogate marker for abdominal obesity. While carrying excess abdominal adiposity is associated with increased CMD risk, it is important to highlight that lifestyle-induced reductions in WC with or without weight loss are associated with improvements in cardiometabolic risk factors. Furthermore, these observations remain consistent regardless of energy restriction or increased EE, highlighting the critical role of reducing WC and thus abdominal adiposity through lifestyle behaviours (Ross et al., 2020).

2.3.2 Non-modifiable Risk Factors

2.3.2.1 Age

Defined by a series of functional and morphological changes that occurs progressively, advancing age is also associated with the deterioration of biological function following its peak reproductive potential. Age plays a vital role in the degradation of cardiovascular (Rodgers et al., 2019) and metabolic function (Palmer & Jensen, 2022), contributing to elevated risk of metabolic disease and CVD in older adults. The prevalence of CVD has been investigated across middle-aged and elderly groups. According to the AHA, they report the incidence of CVD in both men and women to be approximately 54%, 78% and 90% for age ranges 40 – 59, 60 – 79 and ≥80 years

respectively (Benjamin et al., 2019). Age-related cardiovascular changes have been characterised in ageing adults, including systolic and diastolic dysfunction, development of arrythmias (irregular heartbeat), and electrical dysfunction. These functional and electrical abnormalities are precursors to CVD progression, leading to increased prevalence of heart failure, atrial fibrillation and other CVD conditions (Steenman & Lande, 2017).

Ageing is associated with weight gain and changes in body composition. Between the ages of 40 and 66, body weight in both men and women increase at an average rate of 0.3 to 0.5 kg annually (Distefano & Goodpaster, 2018). Additionally, body fat increase by an average of 1% per year (Kuk et al., 2009), with a decline in skeletal muscle mass of 3 – 8% per decade after age 30 years (Volpi et al., 2004). With increasing age, resting EE decline by approximately 4 kcal/year which is closely linked with the decline in lean mass (Anthanont & Jensen, 2016; Frisard et al., 2007; Vaughan et al., 1991). Concomitantly, the thermic effect of food, which is the elevated EE following meals due to the cost of digestion and absorption, is reduced by 1% in older adults when compared to younger adults (Du et al., 2014). Combined with lower levels of physical activity on average performed with increasing age (Pontzer et al., 2021), these components can contribute to disrupted energy balance, leading to weight gain and obesity.

Abdominal obesity is also prevalent in older individuals despite having a healthy BMI (Lukács et al., 2019). As previously mentioned in section 2.1.5, this accompanies an array of cardiometabolic abnormalities and increases cardiometabolic risk. Development of IR also occurs with age, due to impairments of hepatic gluconeogenesis, adipose lipogenesis, defective skeletal muscle glucose uptake and glycogen synthesis (Shou et al., 2020). Furthermore, an increase in circulatory proinflammatory cytokines have been found to be linked with ageing, thought to be associated with age-associated increase in visceral fat (Sepe et al., 2010). Collectively, these cardiometabolic changes associated with ageing are components of a vicious cycle that contribute to the progression of CMD.

2.3.2.2 Sex

There are sexual dimorphisms in ageing and its associated risks with MetS and CVD due to the variations in the rate of decline of sexual hormones (Bhupathy et al., 2010). Sex steroid hormones play a crucial role in influencing prevalence, age of onset and outcome of CVD. Due to the protective benefits of oestrogen, pre-menopausal women experience later onset of CVD compared to men, approximately 7 – 10 years later (Maas & Appelman, 2010).

Unlike the swift decline in sex hormones observed with the cessation of menstruation in women during reproductive ageing, men experience a gradual and continuous decline (Araujo & Wittert, 2011). This decline in endocrine function includes reduced levels of mainly testosterone, as well as other androgens including dehydroepiandrosterone and oestrogens (Araujo & Wittert, 2011). Declines in testosterone occur at 0.4 – 2% following 30 years of age (Wu et al., 2008). Its role as the main androgen, testosterone have protective properties in cardiovascular and metabolic health. Meta-analyses by Corona *et al.* highlight the relationship between low endogenous

testosterone with elevated cardiovascular risk (Corona et al., 2011, 2018), with studies showing low levels associated with development of MetS (Kupelian et al., 2006) and T2D (Ding et al., 2006).

Numerous studies show sexual dimorphism in the prevalence of CMD risk (Gerdts & Regitz-Zagrosek, 2019). For diabetes, there is a higher prevalence amongst men (9.1%) compared to women (8.4%) (Cho et al., 2018). Despite lower risk of CHD in women compared to men, this effect is diminished in the presence of T2D. Women with T2D were found to have a 27% and 44% higher RR of stroke and CHD, respectively, than men (Peters et al., 2014). Additionally, the Strong Heart Study (SHS) in Native Americans found higher prevalence of MetS in women (57%) than men (44%) aged 40 – 49 years (Schumacher et al., 2008). Several cohort studies in different global regions collectively found higher prevalence of preclinical cardiac disease in women. For example, the SHS (De Simone et al., 2011) and Framingham Heart Study (Halland et al., 2018; Lee et al., 2009) in the US, Fat-associated Cardiovascular Dysfunction study (FATCOR) (Halland et al., 2018) in Norway, and Campania Salute Network Project (Gerdts et al., 2018) in Italy, are just some studies that show higher prevalence of left ventricular hypertrophy (De Simone et al., 2011; Gerdts et al., 2018; Halland et al., 2018) and heart failure with preserved ejection fraction in women (Lee et al., 2009), compared to men. Furthermore, the Netherlands Epidemiology of Obesity study found the association between VAT and cardiometabolic risk factors to be higher in obese middle-aged women than in men (Elffers et al., 2017), emphasizing the detrimental effects of VAT in women. It is evident that the lack of oestrogen during the menopause as a result of ageing predispose women to increased CMD risk, highlighting concerns relating to this population.

2.4 The Menopause

2.4.1 Definition of the Menopause

Menopause is a biological consequence of ovarian ageing which signifies the end of reproductivity in women, characterised by the complete depletion of finite ovarian follicles and a sharp attenuation in ovarian oestrogen production (Lambrinoudaki et al., 2021). Oestrogen comprises of three oestrogenic hormones: oestrone (E1), oestradiol (E2), and oestriol (E3), with E2 being the most potent and abundant oestrogen during a woman's reproductive years. In this thesis, reference to oestrogen refers to all composition of the three hormones. Menopause is defined by the permanent cessation of menstrual cycles, identified as twelve months after the last menstrual period (Davis et al., 2015). The average age of natural menopause in the western world is approximately 50 years. Depending on sociodemographic, genetic and lifestyle factors, this age can vary between 45 and 55 years (Ceylan & Özerdoğan, 2015). Menopause before 45 years of age is defined as early menopause, and this occurrence before 40 years of age is defined as premature ovarian insufficiency (POI) (Lambrinoudaki et al., 2021).

2.4.2 Menopause Physiology

Ovarian ageing is a continuous process that occurs in stages, preceding the final menstrual period. This is known as the menopause transition, or perimenopause. As women transition from reproductive age to perimenopause, complex endocrine changes occur that varies across all females, which are accompanied by changes in menstrual patterns and fluctuating reproductive hormones. However, the decline in ovarian follicle numbers is the basis of reproductive ageing. In menstruating women, the intricate relationship between the hypothalamic-pituitary axis and the ovaries maintains the menstrual cycle (Figure 2.2). Briefly, gonadotrophins (luteinising hormone (LH) and follicular stimulating hormone (FSH) are released from the anterior pituitary gland via the stimulation of gonadotropin releasing hormone (GnRH). LH and FSH regulate the secretion of ovarian sex hormones (E2, progesterone and testosterone) and peptide hormones (inhibin A and B). Congruently, an indicator of ovarian reserve known as anti-mullerian hormone (AMH) produced by the granulosa cells of the ovarian follicles, are secreted by the ovaries, independent of the gonadotrophins. During the menopause transition at the end of reproductive age, the rate of attrition of ovarian follicles increases due to the decline in inhibin B secretion from the ovaries. Consequently, the decrease in inhibin B prompts the anterior pituitary to increase FSH secretion, resulting in the stimulation of ovarian E2 production. However, due to the continuous diminishing supply of follicles, maintaining response to the consistent elevated FSH levels of the ovaries declines, leading to reduced E2 output. Eventually, ovarian E2 production ceases with complete loss of functioning follicles, accompanied by sustained high levels of FSH. Similarly, levels of AMH gradually declines throughout the years preceding menopause until undetectable levels are reached (Rajpert-De Meyts et al., 1999). Menopause also causes an increase in androgen to oestrogen ratio with an accompanying decrease in sex hormone binding globulin (SHBG) due to an attenuated oestrogen-liver interaction (Hammond, 2016), ultimately inducing unfavourable pathophysiological manifestations, including a metabolically unhealthy profile (Armeni & Lambrinoudaki, 2022).

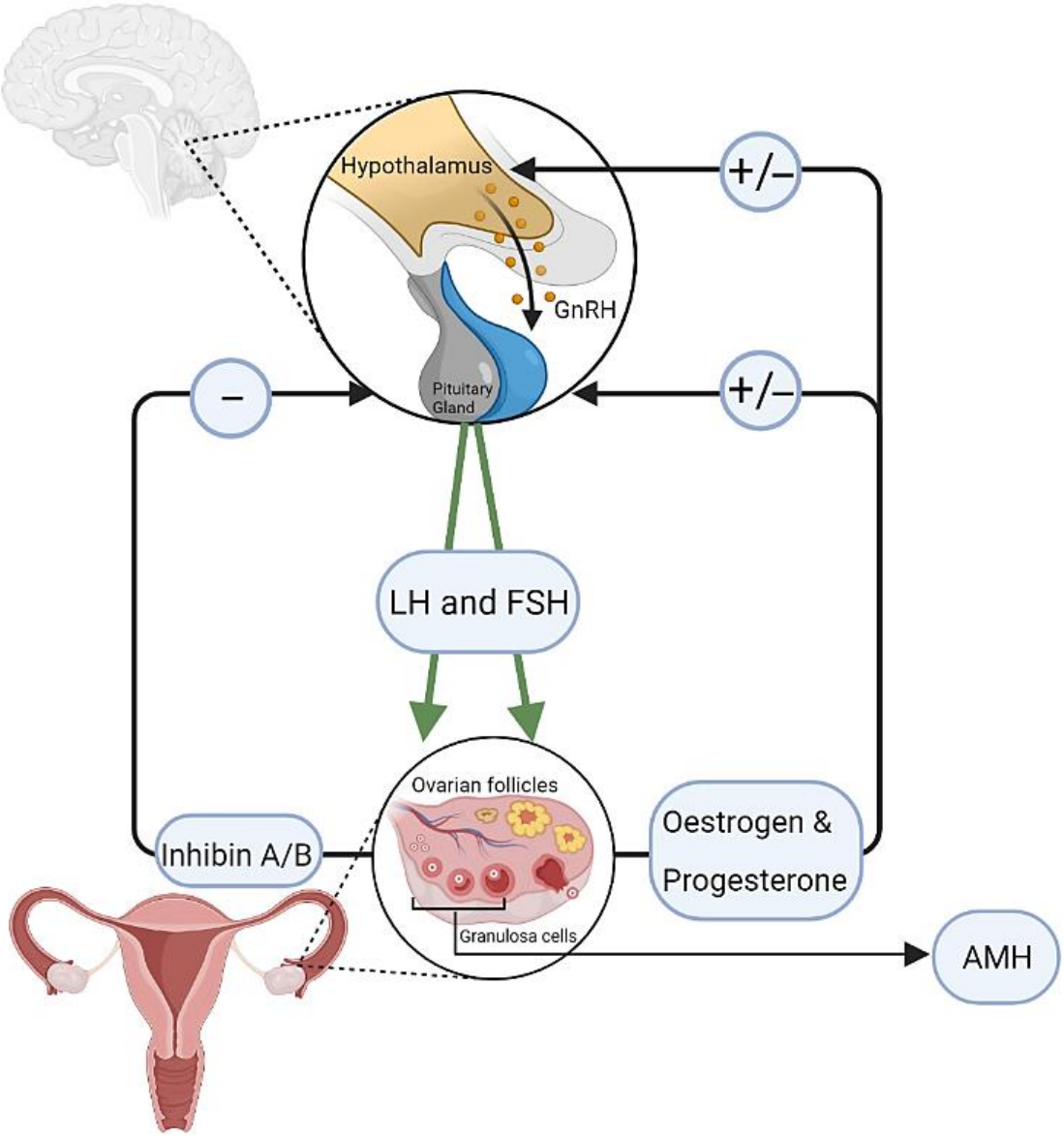

Figure 2.2 Representation of the hypothalomo-pituitary-gonadal (HPG) axis. The hypothalamus secretes gonadotrophin-releasing hormone (GnRH), stimulating the anterior pituitary gland to release gonadotrophins – luteinising hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH act on the ovaries to produce oestrogen, progesterone and inhibins A and B, which inhibits the secretion of gonadotrophins in a negative feedback manner. Independent of the HPF axis, the anti-mullerian hormone (AMH) is a protein hormone secreted by the granulosa cells of the ovarian follicles, specifically the small pre-antral and early antral follicles. ***(Own image).***

The Stages of Reproductive Aging Workshop +10 (STRAW+10) (Harlow et al., 2012) provides a comprehensive staging system to identify the reproductive phases of a woman (i.e reproductive, menopause transition, post-menopause). The stages from perimenopause to post-menopause is depicted in Figure 2.3. The duration of the menopause transition is variable dependent on the individual and can be divided into early or late perimenopause. Early perimenopause is identified by the increased variability of menstrual cycle length of 7 days or more, elevated FSH levels and low levels of AMH, inhibin B and follicle count. In late perimenopause, cycles of amenorrhea last over 60 days accompanied by a steady state of FSH levels of >25 mIU/mL, low levels of AMH and inhibin B. Perimenopause ceases at the end of twelve consecutive months of amenorrhoea, termed menopause. Finally, the stage of post-menopause which can be divided into early and late phases, represents the time spent until death. FSH concentrations continue to rise until levels plateau, while AMH and inhibin B concentrations decreases to undetectable levels (Harlow et al., 2012). In this thesis, menstruating women will be referred to as ‘pre-menopausal women’, while those who have not had a menstrual period for twelve consecutive months will be referred to as ‘post-menopausal women’.

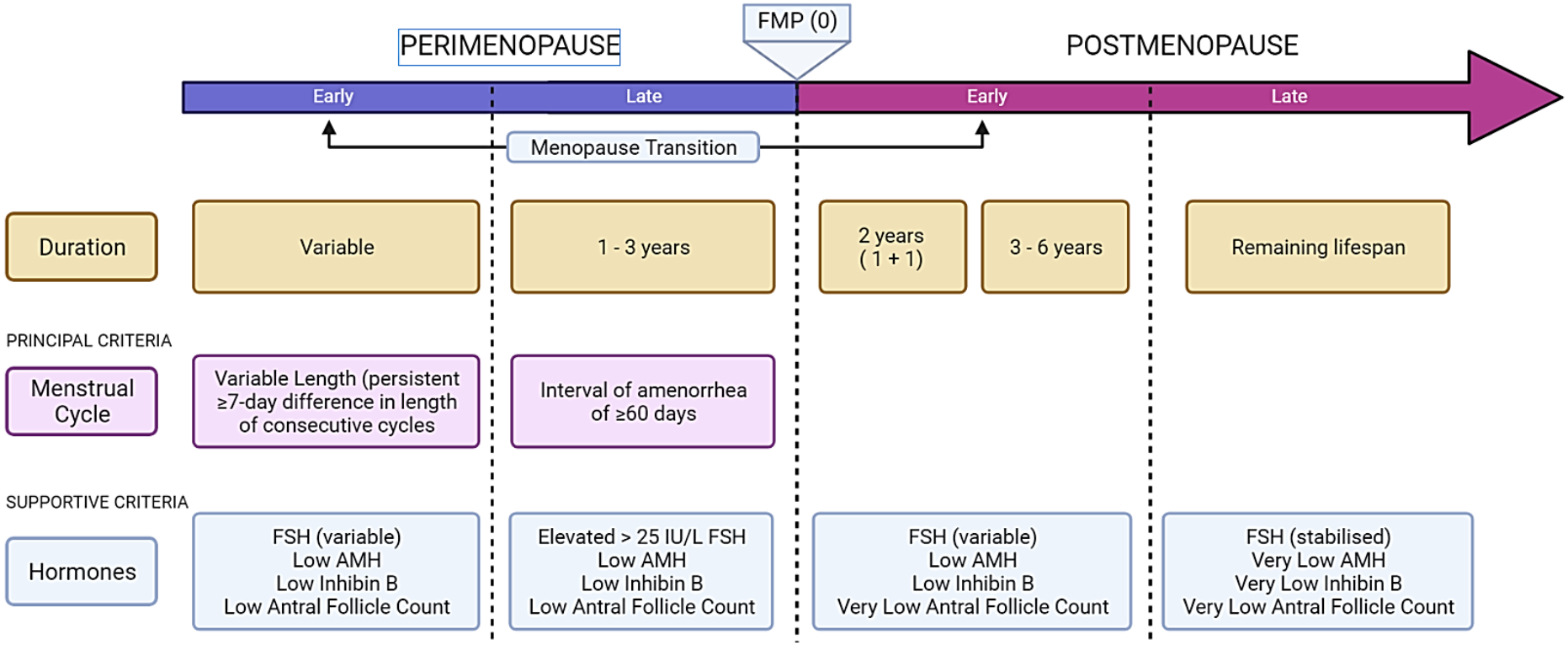

Figure 2.3 STRAW+10 Staging system: a visual representation of the stages of the menopause transition from perimenopause to post-menopause and its associating changes in hormones. FSH: follicle stimulating hormone; FMP: final menstrual period; AMH: anti-mullerian hormone. This figure is a modification adapted from Harlow *et al.* (Harlow et al., 2012).

2.4.3 Cardiometabolic Changes Associated with the Menopause

CVD risk in women surges at midlife (40 – 65 years of age) which coincides with the menopause transition (Shaw et al., 2017). Furthermore, the prevalence of MetS after menopause has been estimated at 31 – 55%, a percentage that is markedly higher in comparison to premenopausal women, independent of age (Stefanska et al., 2015). Post-menopausal oestrogen deficiency accentuates metabolic dysfunction, via adipose tissue redistribution resulting in increased abdominal adiposity (Lovejoy et al., 2008). Additionally, the changes in hormonal milieu and loss of the protective role of oestrogens (especially E2) during the menopausal transition are associated with weight gain, dysregulated lipid profiles and increased blood glucose levels (Karvinen et al., 2019). The metabolic disturbances associated with the menopause phase including hypertension, dyslipidaemia, dysglycaemia and increased VAT accumulation can impede normal endothelial function and accelerate vascular ageing, contributing to increased CVD risk (Moreau & Hildreth, 2014) (Figure 2.4). Albeit an inevitable part of a woman's life, the cumulative effects of ageing and menopause can affect health and quality of life, therefore highlighting the need for concern within this population.

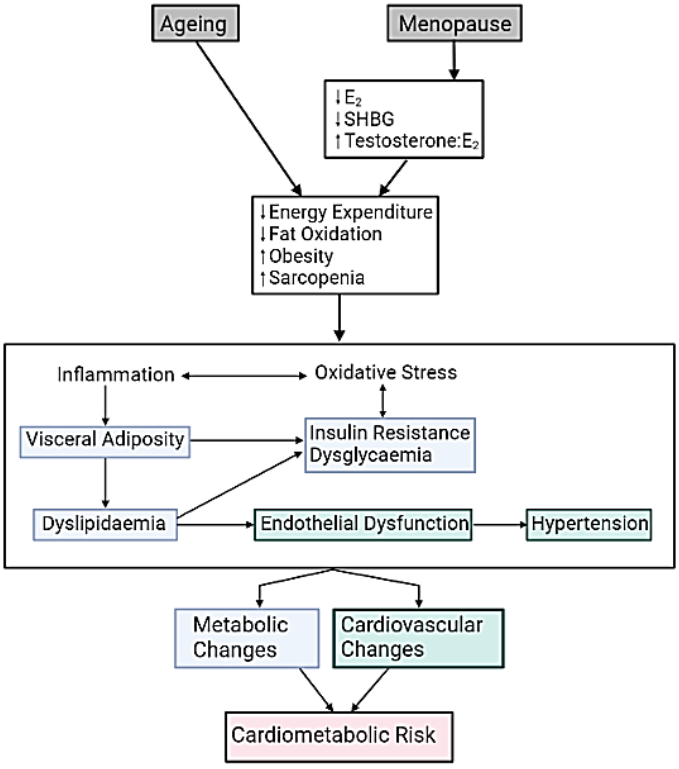

Figure 2.4 Summary of the pathophysiological metabolic and cardiovascular changes associated with ageing and the menopause that contribute to increased cardiometabolic risk. Cardiometabolic risk is exacerbated in women going through the menopause through a synergistic contribution of changes in hormonal milieu including the increase in testosterone to oestradiol (E2) ratio, decrease in E2 and sex hormone binding globulin (SHBG), as well as age-related the changes in body composition, decline in energy expenditure and fat oxidation. Visceral adiposity, dyslipidaemia, IR and dysglycaemia contribute to metabolic dysfunction, while endothelial dysfunction and hypertension result in decline in cardiovascular health. ***(Own image).***

2.4.3.1 Adiposity Redistribution

The redistribution of adiposity to the abdominal area is an important risk factor for the prevalence of cardiometabolic risk in women going through the menopause (Elffers et al., 2017). Longitudinal studies like the Study of Women's Health Across the Nation (SWAN) study (Greendale et al., 2019; Sowers et al., 2007), Avon Longitudinal Study of Parents and Children (ALSPAC) (Clayton et al., 2022), Estrogenic Regulation of Muscle Apoptosis (ERMA) (Juppi et al., 2022), Healthy Transitions study (Marlatt et al., 2020), Montreal-Ottawa New Emerging Team (MONET) study (Abdulnour et al., 2012), Melbourne Women's Midlife Health Project (Guthrie et al., 2010) and the Massachusetts Women's Health Study (MWHS) (Crawford et al., 2000) provide insights into the change in body composition in women throughout the years during their menopause transition. The MWHS longitudinal study and a meta-analysis by Ambikairajah *et al.* reported that confounding factors such as ageing, rather than the menopause transition, was the predominant factor for the increase in fat mass (Ambikairajah et al., 2019; Crawford et al., 2000). However, a consensus from other longitudinal studies including the SWAN study (n = 1246), ALSPAC (n = 1702) and ERMA study (n = 230) demonstrated that the menopause transition is directly associated with increase in fat mass and simultaneous loss in lean mass, independent of age (Clayton et al., 2022; Greendale et al., 2019; Juppi et al., 2022). Particularly, the increase in adiposity was attributed towards the abdominal area (Juppi et al., 2022). The SWAN study found an increase in 5.7 cm in WC following a 6-year observation during the menopause transition (Sowers et al., 2007) and the ERMA study reported a 2.5 kg increase in android fat mass over a 4-year period (Juppi et al., 2022). In support, not only did the Healthy Transitions study (n = 161) found an increase in waist circumference (3.3 cm), visceral adiposity (8.7 cm^2) and abdominal subcutaneous adiposity (8.5 cm^2) (Marlatt et al., 2020) over a 5-year period, but also found significant decreases in serum E2. As previously mentioned in section 2.1.4, the risk of every 1 cm increase in WC coincides with a 5% increased risk of T2D, highlighting the risk associated with excess abdominal adiposity in these women.

The increase in central adiposity and body fat redistribution have been ascribed to alterations in adipose tissue metabolism owing to attenuated oestrogen, with post-menopausal women indicated to have 49% greater intra-abdominal fat area than pre-menopausal women (Toth et al., 2000). Circulating oestrogens play a role in regulating adipose tissue expansion through hyperplasia or hypertrophy. In premenopausal females where oestrogen is abundant, hyperplastic SAT growth is promoted. Conversely in post-menopausal females, hypertrophic VAT expansion is favoured in response to reduced oestrogen levels, resulting in a metabolically unhealthy state (Steiner & Berry, 2022). While there are no generally accepted reference values for VAT, the Tromsø Study in middle-aged adults of European origin found the threshold for VAT mass for MetS prediction in women to be ≥1134g, a value that was also associated with odds ratio for MetS of 3.63 (Lundblad et al., 2021). Furthermore, oestrogen has been shown to influence lipolysis and LPL activity of adipose tissue. Rebuffé-Scrive *et al.* (Rebuffé-Scrive et al., 1986) demonstrated that accumulation of adipose tissue in the gluteal-femoral region is favoured over the abdomen in

premenopausal women, due to the LPL activity and lower lipolytic responsiveness of the adipocytes in the gluteal-femoral area (Arner et al., 1991). Contrastingly, this was not evident in oestrogen-deficient, postmenopausal women as abdominal adipocytes exhibit loss of higher lipolytic rate after menopause, contributing to the predisposition of fat gain in this depot.

2.4.3.2 Reduced Metabolism

Reductions in EE and fat oxidation during post-menopause have been proposed to contribute to changes in body composition (Lovejoy et al., 2008), independent of changes in energy intake (EI) (Mauvais-Jarvis et al., 2017). Reductions in EE during the menopause transition have been found to be associated with decreased physical activity and increased sedentary behaviour, which can result in attenuated basal metabolism, accelerated sarcopenia and muscle atrophy (Geraci et al., 2021). Specifically, a decrease in 0.5% of lean body mass annually has been observed (Greendale et al., 2019). The Healthy Transition study evidenced that post-menopausal women had a 1.5-fold greater reduction in 24-hour EE and in sleep EE, in addition to a 32% decrease in fat oxidation when compared to pre-menopausal women (Lovejoy et al., 2008). Similarly, the MONET study found a progressive reduction in time spent performing moderate physical activity during the menopause transition, possibly explaining the approximate reduction of total EE by 200 kcal/day (Duval et al., 2013). In female rodents, oestrogen modulates EI (Mauvais-Jarvis et al., 2013) and increases EE during physical activity (Krause et al., 2021). Similarly in humans, suppression of sex hormones via gonadotropin releasing hormone agonist therapy for five months results in reduced resting, exercise and total EE in pre-menopausal women. This effect was subsequently ablated following E2 hormonal therapy (Melanson et al., 2015). Collectively, these studies reiterate that the loss of ovarian oestrogen contribute to the accumulation of abdominal adiposity, loss of muscle mass and decreased EE.

2.4.3.3 Dyslipidaemia

Sex differences in lipid metabolism is well established. The Framingham Offspring study is the first and one of the largest studies to demonstrate a decreased risk in CVD risk in middle aged women (n = 1692), reporting two-fold higher concentrations of HDL when compared to men (n = 1574) (Freedman et al., 2004). As such, development of dyslipidaemia is 10 to 15 years later in women compared to men (Sutton-Tyrrell et al., 1998), presumed to be attributed by the protective effect of ovarian hormones (Torre et al., 2013). The atherosclerotic protective action of oestrogen is supported by numerous studies. In rodents, depletion of peripheral E2 decreased lipogenesis inhibition, augmented hepatic lipid accumulation and adipose tissue inflammation (Sun et al., 2016). Similarly in humans, the SWAN study reported that menopause-related oestrogen deficiency increases atherosclerosis risk, owing to VAT accumulation (Samargandy et al., 2021) and accompanying sharp increase in total cholesterol, TRG and LDL concentrations following the year of the final menstrual period (El Khoudary et al., 2019). Complementary to this, a meta-analysis of 66 studies in 114,655 women found significantly higher TRG, TC, LDL and TC when comparing post-menopausal to pre-menopausal women (Ambikairajah et al., 2019).

The relationship between endogenous sex hormones and lipid metabolism in women have been demonstrated. In post-menopausal women, higher oestrogens and SHBG levels have been associated to favourable lipid profiles (Khoudary et al., 2014). E2 exerts anti-atherosclerotic effects by upregulating anti-lipolytic α-2A-adrenergic receptor through SAT located ER-α receptor, but not in VAT (Pedersen et al., 2004). Oestrogen also directly suppresses LPL gene transcription and activity through post-transcriptional modifications (Homma et al., 2000). LPL activity converts TRG into FFA, allowing FFA uptake into non-hepatic tissues. Consequently, reducing LPL activity or expression attenuates FFA uptake (Wang & Eckel, 2009). Hormone replacement therapy (HRT) has been proven to improve dyslipidaemia in post-menopausal women. In these women exhibiting hypertriglyceridemia, TRG levels were normalised or reduced in response to oestrogen replacement therapy (ERT) for one year (Lamon-Fava et al., 2010). In support, a meta-analysis of 73 studies found HRT to be beneficial in improving lipid profiles in post-menopausal women, highlighting the protective role of oestrogen through regulating lipid metabolism (Ambikairajah et al., 2019), and has been found to be associated with better body composition profile (Costa et al., 2020). It should be noted that HRT is not the recommended pharmaceutical treatment for dyslipidaemia.

2.4.3.4 Insulin Resistance and Dysglycaemia

The manifestation of dyslipidaemia is also associated with increased risk of T2D. Previous studies found that 53.5% of patients with hypercholesterolaemia exhibited IR (Bonora et al., 1998). Likewise, 67.1% of patients with T2D presented with dyslipidaemia (Yan et al., 2016). In peri-menopausal women, the recent SWAN study reported the bidirectional relationship between TRG with IR, evidencing that the increase in one will elevate the other. Similarly, the opposing effect was also apparent with HDL (Yu et al., 2022). The mechanism proposed has been pointed to the glucose-fatty acid cycle (Randle cycle), where the accompanying elevation of FFA with increased TRG affects glucose uptake and utilisation of the muscles, resulting in augmented IR.

In addition to dyslipidaemia, the menopause-associated shift of adiposity to the visceral area also promotes low-grade systemic inflammation through cytokine production and peripheral IR (Wu & Ballantyne, 2020). In post-menopausal women without obesity, an increase in abdominal adiposity was found to be associated with decreased insulin sensitivity and glucose tolerance (Sites et al., 2000). In support, a study evaluating women across their menopause transition found reductions of insulin sensitivity in women that gained the most abdominal adiposity (Marlatt et al., 2020). Excess VAT disrupts multiple hepatic metabolic pathways, through promotion of hepatic IR owing to the higher rate of lipolysis and the increase in transport of FFA towards the liver (Hanlon & Yuan, 2022).

Menopause-associated IR is not only explained via the increase in abdominal obesity, but also by the change in sex hormones. The state of high androgens and low bioavailability of SHBG production by the liver during menopause further induces IR (Janssen et al., 2010). Furthermore, oestrogen positively regulates insulin action through promoting insulin sensitivity directly through

actions on insulin-sensitive tissues, or indirectly by regulating factors like oxidative stress that contribute to IR (Gupte et al., 2015). This is supported by a recent meta-analysis of 21 studies comparing age-matched women with POI (n = 1573) to women without POI (n = 1762) presented elevated metabolic risk including increase in insulin levels, lipid markers and FBG (Cai et al., 2022). The administration of exogenous oestrogen revealed mechanistic insights into menopause, insulin sensitivity and glucose homeostasis. Several randomised clinical trials including the Women's Health Initiative and the Heart of Oestrogen/Progestin Replacement Study found a significant reduction in T2D risk in post-menopausal women using ERT (Kanaya et al., 2003; Margolis et al., 2004; Mauvais-Jarvis et al., 2017).

The association between T2D, ageing and obesity is well established, with the ageing effect to be evidently prevalent after 40 years of age (Luo et al., 2020). Consequently, individuals older than 70 years with a healthy BMI have similar risk of T2D relative to individuals in their 30s with a BMI ≥35 kg/m^2 (Fazeli et al., 2020). In this regard, the effect of menopause on glucose homeostasis, independent of ageing, have been reviewed extensively. Initial findings from the SWAN study suggested that chronological ageing, as opposed to ovarian ageing, is associated with altered glucose homeostasis (Matthews et al., 2013). However, recent evidence suggests that menopause and ovarian ageing further exacerbates glycaemic dysregulation and risk of T2D (Creţu et al., 2020). As reported by the ZOE PREDICT 1 study, Bermingham *et al.* reports that post-menopausal women exhibit higher FBG, glycated haemoglobin (HbA1c) and systemic inflammation (GlycA), compared to pre-menopausal women (Bermingham et al., 2022). Furthermore, an exacerbation of glycaemic variability (GV) following the menopause transition was reported, with post-menopausal women exhibiting significantly higher GV when compared to age-matched pre-menopausal women (Bermingham et al., 2022). GV plays a central role in assessing and monitoring glycaemic dysregulating over a period of time, with evidence linking to increased CVD risk (Liang et al., 2017). Collectively, the gains in abdominal obesity in tandem with IR and dysglycaemia is attributed to oestrogen deficiency observed during the menopause transition.

2.4.3.5 Hypertension and Endothelial Dysfunction

CVD risk is increased during the menopause attributed by an amalgamation of age-related metabolic changes and direct effects of changes in the hormonal milieu on vasculature tone (Roa-Díaz et al., 2021). The anti-atherosclerotic properties of oestrogen before the menopause protects the vascular walls through exhibiting anti-apoptotic and antioxidant properties, promoting vasodilation and angiogenesis, and inhibiting fibroblast proliferation (Iorga et al., 2017). The role of oestrogen is paramount in maintaining endothelial function via the increase of vascular endothelium NO synthesis. NO exerts vasodilating action by diffusing into the vascular smooth muscle cells, known as endothelium-dependent vasodilation (EDV). Characterised by impaired EDV, endothelial dysfunction is a contributor to vascular ageing, hypertension and progression of atherosclerosis, of which all are major risk factors of CVD development (Rossi et al., 2008).

As a predictor of hypertension (Rossi et al., 2008), endothelial dysfunction begins during perimenopause, accelerating through the menopause transition due to ovarian ageing and prolonged oestrogen deficiency (Moreau et al., 2012). The pathophysiology of hypertension during the menopause transition is multifaceted and remains controversial, although the mechanisms can be pinpointed to the combination of metabolic changes associated with ageing and hormonal changes of the menopause (Tasić et al., 2022). Unsurprisingly, previous studies found the association between oestrogen deficiency and post-menopausal hypertension. The oestrogen to androgen ratio has also been observed to contribute to higher prevalence of hypertension in post-menopausal women. The Multi-Ethnic Study of Atherosclerosis study of 2834 post-menopausal women found higher testosterone to E2 ratio to be associated with elevated risk for CVD-related events, while higher E2 levels were associated with lower risk CHD (Zhao et al., 2018). Improvements in BP were observed following oestrogen hormone therapy, evident by the Women's Health Initiative study in post-menopausal women with hypertension (n = 9332). Following these women over a 16-year period, Jiang *et al.* found significant improvements in SBP with oestrogen hormone therapy that was independent of antihypertensive medication usage (Jiang et al., 2023).

Undoubtedly, postmenopausal oestrogen deficiency accentuates metabolic dysfunction which predisposes T2D and CVD (Mauvais-Jarvis, 2011). The combination of the effects of postmenopausal oestrogen deficiency and other contributory factors of aging including diminished resting metabolism and reduced physical activity, reiterating the need for concern within this population.

2.5 Ghrelin

Ghrelin is an orexigenic (appetite-stimulating) gut hormone that has gained scientific interest in the past two decades due to its association with obesity, MetS, T2D and CVD (Churm et al., 2017; Pulkkinen et al., 2010; Yuan et al., 2021). Discovered by Kojima *et al.* in 1999, this 28-amino acids long peptide with a N-octanoylated serine 3 (Ser3) residue was first reported to be found in rat and human stomachs. Ghrelin acts as an endogenous ligand for the growth hormone secretagogue receptor 1a (GHSR-1a), thus stimulating release of growth hormones (GH). As such, this peptide was named after the Proto-Indo-European root '*ghre*' which means 'grow' (Kojima et al., 1999).

Prior to secretion, ghrelin is synthesised via post-translation from its precursor pre-proghrelin that contains 117-amino acids. In humans, approximately 60 – 70% of ghrelin secretion predominates from the endocrine P/D1-like cells of the oxyntic glands of the fundus mucosa located in the stomach (Ariyasu et al., 2001). The remainder originates from the small intestines, with minor quantities expressed from other organs including the hypothalamus (arcuate nucleus (ARC) and paraventricular nucleus (PVN)), lung, kidneys and pancreatic islet cells (Kojima & Kangawa, 2005).

Synthesis of ghrelin is encoded by the pre-proghrelin gene (*GHRL)* (Figure 2.5). Situated on chromosome 3p25-26, *GHRL* comprises of five exons interspersed with four introns. The transcribed ghrelin messenger RNA (mRNA) undergoes splicing, resulting in a 117-amino acid preproghrelin precursor. Subsequent cleavage processes yield ghrelin and obestatin. Obestatin is a 23-amino acid peptide involved in the regulation of energy balance. In contrast to ghrelin's appetite-stimulating effects, obestatin antagonises the actions of ghrelin, with actions not limited to the suppression of appetite and reducing food intake (Zhang et al., 2005).

2.5.1 Isoforms of Ghrelin and Ghrelin O-acyltransferase

Ghrelin exists in two variants distinct via an acyl moiety: acyl ghrelin (AG) and des-acyl ghrelin (DAG) (Figure 2.5). In circulating fasting plasma of healthy adults, AG constitutes less than 10% of circulating total ghrelin (TG), thus making most of the circulating ghrelin to be DAG (Patterson et al., 2005). Both isoforms of ghrelin exhibit their functions independently and in opposition (Abdalla, 2015). Throughout this thesis, this hormone will be denoted to as ‘ghrelin’ when addressing it comprehensively, and referred to as ‘AG’, ‘DAG’, and ‘TG’ when cited specifically from literature.

The acylation of ghrelin is a post-translational modification at Ser3 catalysed by the enzyme ghrelin O-acyltransferase (GOAT). Belonging to the MBOAT family that contains sixteen enzymes, GOAT is the only enzyme known catalyse the acyl modification of ghrelin. Previously identified as MBOAT4, localisation of GOAT mRNA is broadly expressed in human tissues, including the stomach, pancreas and plasma. GOAT transfers the O-acyl acid to the hydroxyl group of Ser3 of ghrelin. In the ER, GOAT binds n-octanoyl-CoA to proghrelin, where proghrelin is then transported to the Golgi apparatus (Gutierrez et al., 2008). The octanoylation of ghrelin into AG is essential for its binding to the GHSR-1a, thus activating it to secrete GH (Kojima et al., 1999) (Figure 2.5).

In the absence of acylation, ghrelin can be secreted in the form of DAG. DAG is acknowledged as a breakdown product of AG within the circulatory system due to the esterase-catalysed deacylation by multiple plasma proteins, particularly butyrylcholinesterase (Chen et al., 2017) and acyl-protein thioesterase 1 (Satou et al., 2010). Emerging evidence highlights the significant biological activity of DAG, despite the lack of GHSR affinity (Kojima et al., 1999). DAG has shown to antagonise certain orexigenic effects of AG (Fernandez et al., 2016). However, the receptors and mechanisms underlying this antagonistic role is yet to be defined (Cui et al., 2017).

TG is commonly referred to as the ‘hunger hormone’, as implicated in the regulation of energy balance, as well as potential clinical target for obesity and weight management interventions. Although TG concentrations can exhibit diurnal variations, its secretion is influenced by meal timing, macronutrient content, and other factors including age, sex and BMI (Anderson et al., 2023).

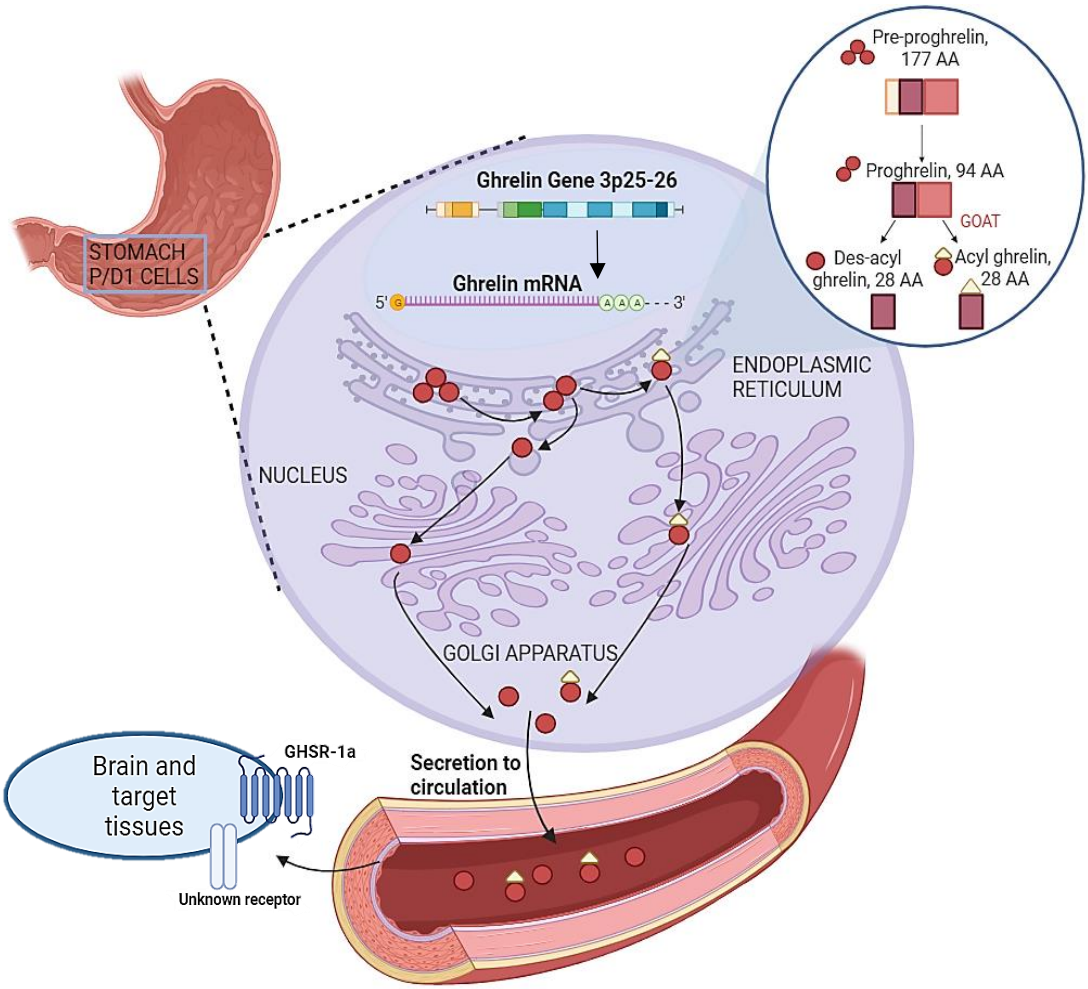

Figure 2.5 Schematic illustrating the post-translational process of ghrelin and the components of the ghrelin system. In humans, ghrelin is predominantly secreted from the P/D1 cells within the gastric mucosa. In the nucleus, the ghrelin gene encodes a 117 amino acid (AA) precursor protein – pre-proghrelin. In the endoplasmic reticulum, signal peptide cleavage yields proghrelin, a 94 AA length peptide, which can be subsequently acylated to acyl ghrelin by the enzyme ghrelin O-acyltransferase (GOAT). Mature forms of acyl ghrelin and un-acylated ghrelin (des-acyl ghrelin) are packaged into secretory vesicles by the Golgi apparatus to be secreted into circulation. Following secretion, acyl ghrelin binds and activates the growth hormone secretagogue receptor 1a (GHSR-1a) in the brain and other target tissues, while des-acyl ghrelin activates its own, yet unidentified, receptor. ***(Own image).***

2.5.2 Physiological Functions

As previously mentioned, ghrelin is a hunger hormone. The physiological functions of ghrelin include stimulating appetite, increasing gastric mobility and gastric acid secretion, as well as energy metabolism (Kojima et al., 1999). Additionally, it also exhibits multi-faceted roles including the regulation of glucose homeostasis, lipid metabolism, cardiovascular system and inflammation (Figure 2.6) (Sovetkina et al., 2020).

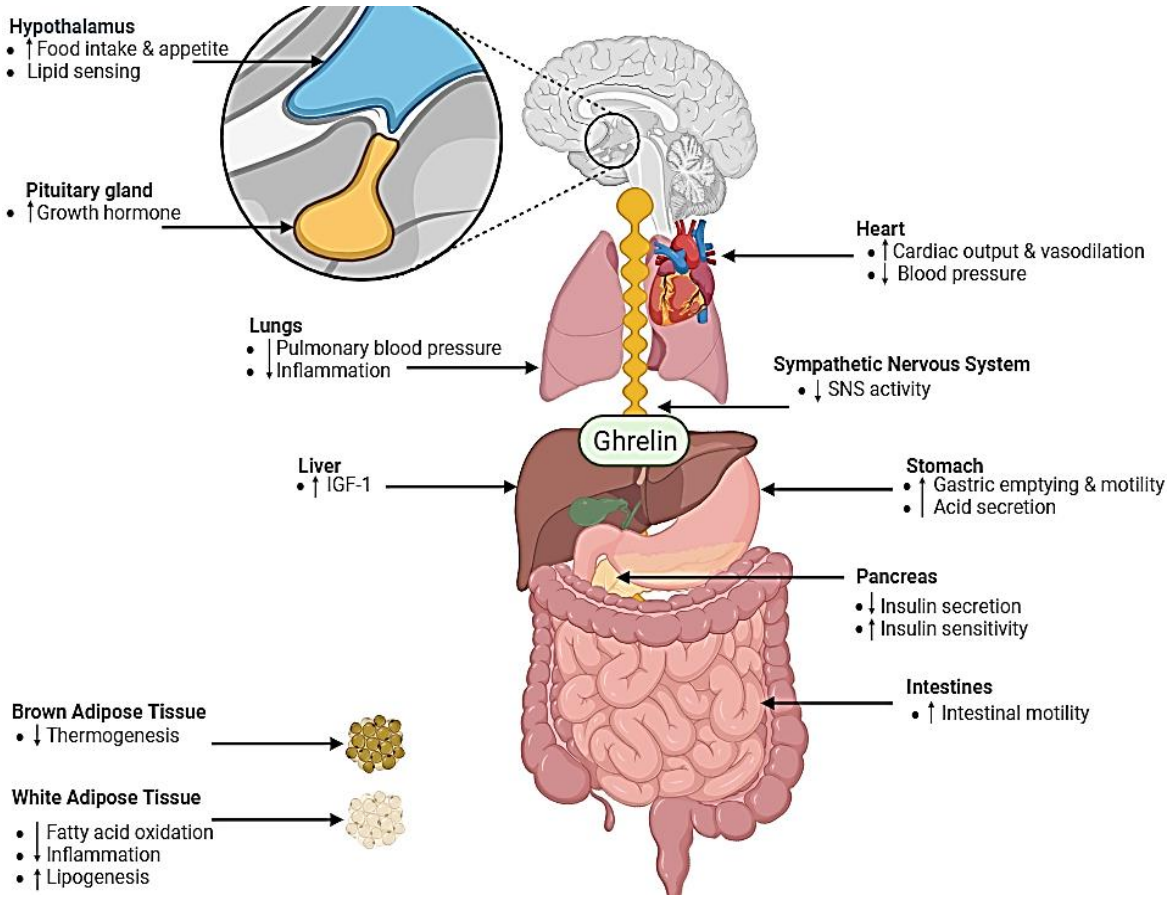

Figure 2.6 Summary of the physiological effects of ghrelin. IGF-1: insulin-like growth factor-1; SNS: sympathetic nervous system. Up and down arrows denote increase and decrease, respectively. Diagram adapted from Akalu et al., 2020 (Akalu et al., 2020).

2.5.2.1 Orexigenic Effect and Energy Homeostasis

To date, ghrelin exists as the only gut hormone with an orexigenic role. Ghrelin is involved in short-term regulation of food intake and plays an important role in appetite and meal initiation. In response to hunger and starvation, ghrelin produced primarily by the stomach enters the blood circulation. The stimulation of gastric motility through gastric emptying that signifies the sensation of hunger have been observed with high doses of ghrelin infusion in humans. The rise in circulatory ghrelin serves as a peripheral signal to the central nervous system (CNS) to activate specific pathways to stimulate feeding (Müller et al., 2015).

It is well known that food intake and appetite is controlled by complex pathways in the CNS and the brain, specifically the hypothalamic region. In particular, the ARC of the hypothalamus regulates feeding and metabolism (Myers & Olson, 2012). It contains two neuronal populations that are responsible for the opposing effects of food intake: pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript neurons that suppress feeding, and neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons that stimulate feeding. AG initiates feeding activity by crossing the blood-brain barrier to stimulate NPY/AgRP neurons that promotes secretion of NPY and AgRP peptides, which in turn signals the PVN to initiate feeding (Cowley et al., 2003), In

addition, AG's orexigenic action is mediated through GHSR-1a, which mainly exists in ARC. Pre-prandial secretion of TG is the highest prior to an anticipated meal (Cummings et al., 2001), whilst TG levels are suppressed postprandially, with strongest effect following a protein-rich or carbohydrate-rich meals (Foster-Schubert et al., 2008). Additionally, AG administration induces a shift in food preference towards fatty diets, as well as divert from the metabolic utilisation of fat, potentially facilitating fat storage (Müller et al., 2015). This shift is accompanied by the engagement of various eating behaviours, including heightened feeding responses to visual cues, conditioned preference for food rewards in specific locations, and increased locomotor activity in anticipation of a chocolate reward (Müller et al., 2015).

Consistent with its orexigenic mechanism of action, ghrelin has accountability in the control of energy homeostasis (Al Massadi et al., 2017). Energy homeostasis is the coordinated regulation between EI and EE. In line with this, different metabolic statuses can affect the synthesis, secretion and degradation of ghrelin (Yin et al., 2009). Ghrelin's orexigenic effects increase EI which subsequently induces body weight gain and adipogenesis (Wiedmer et al., 2007). Additionally, ghrelin plays a role in long-term body mass modulation. Plasma ghrelin levels fluctuate to compensate during body weight changes. For example, AG levels decrease with weight gain during conditions such as pregnancy (Palik et al., 2007), overfeeding (Williams et al., 2006) or during a high-fat diet (Otukonyong et al., 2005). Conversely, AG levels augment with weight loss during conditions such as long-term chronic exercise (Kraemer & Castracane, 2007), starvation (Purnell et al., 2007), or cachexia from anorexia nervosa (Soriano-Guillén et al., 2004). For the purpose of the thesis, mechanisms of conditions associated with overnutrition including obesity, MetS and CVD will be further elaborated from sections 2.5.3.

2.5.2.2 Lipid and Adipocyte Metabolism

Ghrelin also exhibits adipogenic and lipogenic properties. It has been suggested that ghrelin exerts an autocrine/paracrine effect on human adipose tissue as the components of the ghrelin system; ghrelin, GOAT, GHSR-1a, are expressed in these tissues. Ageing accompanies increase in WAT as well as declines in BAT and its thermogenic function, contributing to downstream age-associated comorbidities including adipose tissue inflammation (Nirengi & Stanford, 2023). Understanding the ghrelin signalling pathway in WAT and BAT can aid in better understanding the complexities of ghrelin's role in metabolic regulation. Macrophages are important mediators of adipose tissue inflammation. In ageing mice, Lin *et al.* found that ablation of GHS-R promote macrophage polarisation in WAT and BAT that favours an anti-inflammatory and less IR profile. Specifically, GHS-R ablation promoted lipid mobilisation by releasing FFA in WAT and thermogenesis in BAT, fostering a lean and insulin sensitive metabolic state (Lin et al., 2016).

Through activation of AMPK, a key regulator of cellular energy balance, AG promotes adipogenesis, thereby contributing to *GHRL* (Gurriarán-Rodríguez et al., 2011). Furthermore, AG promotes adipocyte differentiation through stimulation of adipogenic transcription factors sterol regulatory element-binding transcription factor 1 and PPARγ (Rodríguez et al., 2009). Additionally

in VAT, AG and DAG stimulate lipid accumulation through activating the expression of fat storage-related proteins such as acetyl-CoA carboxylase, LPL and fatty acid synthase in adipocytes (Rodríguez et al., 2009). Ghrelin also inhibits sympathetic efferents to BAT and impedes the intracellular breakdown of TRG into FFA (Yasuda et al., 2003), resulting in weight gain.

By increasing expression of key adipogenic genes, ghrelin promotes adipogenesis by stimulating the differentiation of preadipocytes into mature adipocytes (Rodríguez et al., 2009). Furthermore, ghrelin can increase lipid retention by decreasing the transcription of genes involved in cholesterol efflux, thereby inhibiting lipolysis and contributing to increase in adipocyte volume (Davies et al., 2009). *Ex vivo,* this increase in expression of lipogenic and adipogenic transcription factors has been observed in human visceral adipocytes following AG and DAG treatment (Rodríguez et al., 2009). Chronic AG administration in humans have shown to increase body fat content (Tschop et al., 2000). Ghrelin can stimulate lipogenesis and suppress lipid oxidation specifically in white adipocytes. Conversely, in brown adipocytes, the infusion of central ghrelin leads to a reduction in the expression of uncoupling proteins, crucial molecules contributing to energy dissipation and thermogenesis (Thompson et al., 2004).

2.5.2.3 Cardiovascular System

Ghrelin possesses diverse cardiovascular effects, owing to the expression of ghrelin receptors in various tissues of the cardiovascular system, including the heart, myocardial and vascular smooth muscle cells (Gnanapavan et al., 2002). In recent years, studies have shown that ghrelin exerts cardioprotective effects, including the enhancement of vascular activity and angiogenesis, modulating sympathetic activity and hypertension, inhibiting arrythmias and reducing heart failure (Tokudome & Kangawa, 2019). By regulating intracellular calcium concentrations, ghrelin improves cardiac performance (Colldén et al., 2017). Additionally, AG can suppress cardiac sympathetic nerve activity due to its effect on electrical activity of the heart as well as stimulating cardiac parasympathetic nerve activity (Soeki et al., 2014). DAG has also been found to inhibit excessive collagen deposition on the heart, a characteristic of cardiac fibrosis, thereby protecting against cardiac dysfunction (Pei et al., 2015).

AG is also a potent vasodilator, leading to a decrease in mean arterial pressure without alteration of heart rate in healthy humans (Ukkola, 2015). Additionally, AG demonstrates inhibitory effects on the production of proinflammatory cytokines in human endothelial cells (Ruolan, 2017), enhances endothelial function (Tesauro et al., 2005), suppresses vascular smooth muscle cell proliferation (Shu et al., 2013), and mitigates atherosclerosis by inhibiting ER stress (Ai et al., 2017). As such, it can inhibit the formation of atherosclerotic plaque, thereby promoting plaque stability (Ukkola, 2015). The action of vasodilation via AG can occur in a NO-independent mechanisms by inhibiting the SNS, consequently reducing circulatory levels of nor-epinephrine (Okumura et al., 2002), a potent vasoconstrictor. On the other hand, DAG can induce vasodilation via NO-dependent mechanisms in endothelial cells and in intact vessels (Togliatto et al., 2010) through the phosphorylation of endothelial nitric oxide synthase (eNOS) (Togliatto et al., 2010),

GHSR-mediated signal pathways of AMPK and Akt (Dimmeler et al., 1999; Fulton et al., 1999), one of the main kinases involved also in insulin signalling pathway.

Ghrelin can also play an important role in BP regulation and circulating AG have been found to be inversely correlated with both SBP and DBP (Mao et al., 2016). In female adults, individuals with hypertension (≥140/90 mmHg) exhibited lower levels of circulatory AG, irrespective of abdominal obesity (Yu et al., 2018). Multiple studies in healthy humans revealed that AG administration decreased cardiac sympathetic nerve activity and BP by both NO-dependent and NO-independent mechanisms, thus promoting peripheral vasodilation (Soeki et al., 2014). Interestingly, this response was not evident following DAG administration, suggesting that activation of the ghrelin receptor via AG is required to elicit these autonomic effects (Zhang et al., 2017).

2.5.3 Ghrelin and Cardiometabolic Disease

Due to its recognised role in energy, lipid and glucose metabolism, the investigation of the ghrelin pathway as a therapeutic target for addressing disorders related to overnutrition has gained considerable amount of interest. Ghrelin has shown to be associated with obesity, T2D and MetS (Pulkkinen et al., 2010). Despite its orexigenic properties, individuals with these conditions exhibit a paradoxical decrease in fasting circulating AG and DAG levels. The following subsections aim to discuss the relevant literature on the role of ghrelin in the pathophysiology of obesity, MetS and CVD.

2.5.3.1 Obesity

In comparison to lean individuals, individuals with obesity exhibit lower basal AG and DAG levels due to a physical compensatory adaptation to a chronic state of energy surplus (Wang et al., 2022). These metabolic adaptations serve as a protective mechanism to preserve the elevated body weight set point established during obesity, as well as mitigating weight loss by attenuating resting metabolic rate (Fothergill et al., 2016) and altering circulating levels of hormones associated with energy balance (Briggs et al., 2010). As such, ghrelin resistance can occur in individuals with diet-induced obesity (DIO) to impair ghrelin's functions in homeostatic feeding and reward processing, thereby preventing the further increase of the achieved higher bodyweight set point (Zigman et al., 2016). In DIO, ghrelin secretion in the stomach is impaired, where ghrelin-secreting cells no longer respond effectively to the stimulatory actions of nor-epinephrine or the inhibitory actions of glucose (Uchida et al., 2014). Fluctuations in ghrelin levels relating to mealtimes are blunted in DIO individuals, where feeding failed to diminish AG levels within this population (English et al., 2002). This results in attenuated circulating plasma ghrelin and impaired post-prandial ghrelin levels. It was hypothesised that ghrelin resistance in DIO stems from ghrelin resistance of the arcuate NPY/AgRP neurons found in the hypothalamic circuitry that controls food (Briggs et al., 2010). DIO reduces ghrelin transport across the blood-brain barrier, impairing the neural circuits in the hypothalamus responsible for feeding mechanism (Banks et al., 2008). To restore ghrelin sensitivity, diet-induced weight loss can reverse the attenuated circulating

ghrelin levels caused by obesity (Briggs et al., 2013). Consequently, the restoration of ghrelin function induced by weight loss contributes to the replenishment of energy reserves, thus facilitating weight regain (Zigman et al., 2016).

There are also distinctions in fasting and postprandial ghrelin concentrations observed in populations without diabetes as well as between lean and obese individuals. In normal-weight individuals, postprandial plasma ghrelin levels were suppressed in proportion to the calorie content of the meal (Tschöp et al., 2001). However, this regulatory pattern was not mirrored in obese subjects (English et al., 2002), indicating that food intake may not effectively suppress ghrelin levels in individuals with obesity (Higgins et al., 2007).

2.5.3.2 Metabolic Syndrome

Reduced ghrelin concentrations have also been found to be linked to an increased prevalence of MetS, with progressively diminishing ghrelin levels correlating with the number of components comprising the syndrome (Ukkola, 2009). This association is primarily attributed to a higher BMI in individuals with lower ghrelin levels, since adiposity significantly influences all other MetS features (Pulkkinen et al., 2010; Ukkola, 2009). Obesity and its related comorbidities are also related to IR. As a signal of positive energy, the increase in FBG stimulates insulin secretion, further suppressing ghrelin secretion (Yanagi et al., 2018). In middle-aged (Ukkola et al., 2006) and elderly (Langenberg et al., 2005; Serra-Prat et al., 2009) individuals with MetS, fasting TG levels are attenuated compared to individuals of similar age without MetS. Additionally, DAG concentrations have been found to be lower in obese individuals with MetS compared to their nonobese counterparts (McLaughlin et al., 2004).

As obesity is associated with decreased circulating ghrelin as well as the pathophysiology of MetS, studies have explored the administration of exogenous ghrelin in individuals with obesity-related MetS. Hypertension is one of the features of MetS. In MetS individuals with obesity, AG administration played an important role in restoring the physiological balance between vasoconstrictor and vasodilator forces (Tesauro et al., 2009), beyond its role of regulating food intake. Failure to vasodilate adequately following exposure to endothelium-dependent vasodilators in obesity-related MetS is an indicator of impaired NO bioavailability, as well as excess vasoconstrictor tone, leading to endothelial dysfunction (Kajikawa & Higashi, 2022). Thus, contributing to the maintenance of vascular homeostasis in these individuals. Studies have also shown the involvement of ghrelin in the metabolism of insulin and glucose. AG administration in healthy individuals reduced insulin levels and increased glucose levels (Broglio et al., 2004). Contrastingly, DAG administration improved glucose metabolism and insulin sensitivity (Benso et al., 2012). Individuals with MetS and obesity also present higher AG/DAG ratio compared to non-obese individuals with MetS, suggesting the promotion of IR associated with excessive AG (Barazzoni et al., 2007).

2.5.4 Ghrelin and the Menopause

Women who have gone through the menopause exhibit a large variation of confounding factors including age, adiposity and metabolic health status (as discussed in previous sections), which can have profound effects on ghrelin profiles. To better understand the association of menopause and ghrelin, it is crucial to first comprehend the relationship between ghrelin and oestradiol.

2.5.4.1 Oestradiol

There are clear sex distinctions in the modulation of body weight and EE (Shi & Clegg, 2009) which could be pinpointed to the biological differences in the regulation of food intake. Neuroimaging studies on brain pattern response to hunger, food stimulation and satiation revealed sex-based differences (Cornier et al., 2010). When compared to men, women elicited greater neural response to food-related visual stimuli (Uher et al., 2006). Furthermore, changes in ovarian hormones are responsible for alterations in female's EI during the menstrual cycle. There is a consensus from a recent review that conclude higher EI in the luteal phase (where oestrogen levels are lowest), compared to the follicular phase (where oestrogen levels are highest) (Rogan & Black, 2023). E2 mainly accounts for the sex differences of food intake and energy balance (Butera, 2010), owing to its anorexigenic (appetite-suppressing) effect for the decrease in EI during the follicular phase (Santollo & Daniels, 2019). In line with this, the metabolic effects of E2 opposes to that of ghrelin (Geary & Asarian, 2006). Understanding the relationship between the altered dynamics of both hormones may aid in the better understanding of their dysregulation in the role of metabolic function in menopause.

There are overlaps in regions of the brain where E2 and ghrelin both influence food intake and EE through their receptors (Spary et al., 2009). It has been identified that oestrogen receptors are localised within the ARC and PVN areas, with region specific differences in the subtypes. ARC contains primarily ER-α subtype, while PVN contains primarily ER-β subtype (Merchenthaler et al., 2004). As previously mentioned, ARC and PVN are areas of the brain involved in the regulation of feeding, where the effects of oestrogen on food intake and body weight are mediated via these receptors (Sloan et al., 2018). Early studies in animals show that E2 decreases ghrelin sensitivity (Butera et al., 2014; Clegg et al., 2007). In female rats, exogenous AG promoted food intake. This behaviour was more apparent in ovariectomised (OVX) rats compared to E2 treated ovariectomised or ovarian-intact rats (Butera et al., 2014; Clegg et al., 2007), implying the antagonistic effect of E2 on the orexigenic influences of ghrelin. Reductions of circulating E2 following OVX may alter activity within ARC and PVC either directly or indirectly via the ghrelin pathway, thus affecting feeding regulation exhibited in these studies (Burch et al., 2022). Additionally, ghrelin and ER-α immunoreactivities have been demonstrated to exist in the same cells, suggesting that oestrogen may have a direct effect on ghrelin expression (Matsubara et al., 2004).

There is scarcity in the exploration of ghrelin variation during the menstrual cycle. Despite the relationship between ghrelin and E2, studies that examined ghrelin levels across the menstrual

cycle in pre-menopausal women reported non-significant changes in AG and DAG (Dafopoulos et al., 2009), as well as TG (Salem et al., 2022). Yet, other studies have established the relationship between menstrual irregularities, EI and nutritional status (Miyamoto et al., 2021). It is established that amenorrhea is associated with oestrogen deficiency (Shufelt et al., 2017). Young athletic women with exercise-associated amenorrhoea exhibited elevated ghrelin levels when compared to women that were sedentary or habitually active (De Souza et al., 2004). The observed ghrelin profile in these athletic women is analogous to numerous observations in women with anorexia nervosa (Schalla & Stengel, 2018), even when matched for BMI (Tolle et al., 2003). Similar to the attenuated levels of ghrelin seen in obesity (Wang et al., 2022), elevated ghrelin levels may be an adaptive response to chronic state of nutrition deprivation (Schalla & Stengel, 2018). The chronic state of energy deficit in both anorexia nervosa and athletes could influence the changes in hormones, including ghrelin, leading to menstrual disturbances. However, the paucity of research poses a challenge to fully delineate the mechanisms in this area. Nevertheless, the influence of E2 on ghrelin and food intake regulation should not be overlooked.

2.5.4.2 Menopause

The relationship between ghrelin levels and menopause status is complex, owing to the changes associated with the menopause that have profound effects on ghrelin individually, including diminishing oestrogen levels and features of ageing. Albeit limited, the relationship between ghrelin levels and menopause status have been explored of which results are conflicting. A reduction in circulating AG and TG have been observed in post-menopausal women (Sowers et al., 2008), yet several other studies have reported no association between menopause status and ghrelin (Iwamoto et al., 2005; Karakus et al., 2012; Purnell et al., 2003). Interestingly, a longitudinal study following women across their midlife found AG levels to be significantly associated with multiple CVD risk factors changes (Wildman et al., 2008). This is also supported by another study that found significant inverse correlation between AG levels, body weight and adiposity in healthy post-menopausal women that was absent in the pre-menopausal counterpart (Iwamoto et al., 2005). This suggests that alterations in ghrelin levels following the menopause is indeed more complexed and likely affected by a combination of menopause and ageing.

Given that oestrogen deficiency during the menopause transition increases risk for susceptibility to metabolic dysfunction that may be associated with dysregulated ghrelin, ERT and its effects on ghrelin has been explored. 21-days of HRT with oestrogen improved of hypothalamo-pituitary sensitivity to AG (Kok et al., 2008). Similarly, increases in AG were observed following six months of ERT with significant improvements seen in all components of the lipid profile and DBP (Kellokoski et al., 2005). Further, elevations in AG were significantly correlated with changes in circulating E2 (Kellokoski et al., 2005), suggesting the notion of increasing ghrelin levels with ERT in benefiting cardiovascular risk factors. Meta-analyses have evaluated the benefits of HRT in post-menopausal women in the reduction of MetS and CVD risk factors (Kim et al., 2020; Nudy et al., 2019). However, HRT also exhibit its risk, with timing of initiation and underlying disease to

be important factors for consideration (Kim et al., 2020). Furthermore, NICE guidelines recommend that antidepressants, an alternative non-hormonal therapy to treat menopausal symptoms, should not be routinely prescribed (British Menopause Society, 2022). Thus, other therapeutic routes should be considered.

AG, DAG and GOAT, present appealing targets for the development of pharmacological interventions against progression of MetS and T2D in these women. Pharmaceutical companies have developed drugs capable of targeting the orexigenic or obesity-related functions of ghrelin, its receptor, or GOAT (Schellekens et al., 2010). For example, ghrelin receptor antagonists have demonstrated efficacy in blocking GH secretion, thereby improving diabetic conditions by promoting glucose-dependent insulin secretion, inducing weight loss, and suppressing appetite (Zorrilla et al., 2006).

However, with the rise in the obesity pandemic and increasing numbers of comorbidities, prescription medicine poses a heavy financial burden on the NHS. In the past 15 years, there has been a 42.6% increase in medication prescription rates in the UK, of which the most common prescriptions were cardiovascular related (30.2%) (Naser et al., 2022). Particularly, the most dispensed medications were heart failure and hypertensive related. This is consistent with the escalating trend of CVD prevalence that has nearly doubled from 271 million in 1990 to 523 million in 2019 (Roth et al., 2020). Therefore, lifestyle interventions including exercise and dietary interventions are the first line of defence for preventing and treating metabolic disorders associated with obesity, MetS and CVD.

Chapter 3 – General Methodology

This thesis consists of two experimental studies, each with its own independent research study designs. The following chapter will outline the general methods of data collection employed in Chapter 5 (GHREX): Establishing the effects of a home-based, equipment free high-intensity interval training on cardiometabolic risk markers and feasibility on mediating ghrelin response in post-menopausal women, and Chapter 6 (GHREXD): Investigating the cardiometabolic and ghrelin response to home, equipment free high-intensity training with or without the Mediterranean-style diet in post-menopausal women: a randomised controlled trial. Study specific information for can be found in the appropriate chapters and appendices.

3.1 Participants

All participants recruited were healthy, overweight/obese post-menopausal women between the ages of 45 – 65 years. Post-menopause was defined as not experiencing menstrual periods for ≥12 months due to natural or surgical menopause (Lambrinoudaki et al., 2021). The general inclusion criteria for all studies were that participants had to be non-smokers and have no known diseases. The main exclusion criteria were:

1) not post-menopausal
2) body mass index (BMI) <25 kg/m^2
3) presenting health diseases such as cancer, cardiovascular, kidney, liver, gastrointestinal, diabetes (type 1 or 2)
4) on HRT for <6 months
5) prescribed medications related to cardiometabolic health conditions including statins, metformin, anti-hypertensives or beta-blocker medications

3.2 Recruitment

Ethical approvals were reviewed and favourable ethical opinions for conduct were given from the NHS by the Solihull Research Ethics Committee (REC; IRAS: 288904) for Chapter 5 and Hampstead REC (IRAS: 314505) for Chapter 6. Both studies were conducted in accordance with the Declaration of Helsinki. Participant recruitment methods varied and included advertisements via staff email, intranet, social media, and posters. Recruitment related to Chapter 5 were recruited between April 2021 and October 2021, and from May 2022 to July 2023 for Chapter 6.

Potential participants expressed interest via phone or email. Study procedures were explained verbally and/or subsequently via email which contained information detailing the study description and experimental procedures in the Participant Information Sheet (PIS) (Appendix 1 and 3). The PIS also contained the potential risks and benefits of participation, the preservation of their anonymity, as well as their right to withdraw from the study at any time without any disadvantage to themselves or others. Participants had given written and informed consent prior to participation.

3.3 Standardisation of study visit procedures

All study visits procedures were conducted in the Applied Sports Technology, Exercise and Medicine (A-STEM) Research Centre in Swansea University Bay Campus. Eligible participants attended three separate lab visits in Week 0, 1 and 8, and completed the 8-week HEFHIIT at home. Prior to all visits, participants were instructed to avoid alcohol for 24 hours and fast (except water) for at least 12 hours the night before. Physical activity metrics were monitored with a Garmin Forerunner 35 accelerometer (Garmin Ltd, United States) from Week 0 to Week 8. Participants were instructed to refrain from altering their baseline activity levels.

3.3.1 Overview of Study Visit Procedures: Chapter 5

Following giving informed consent, the screening visit at week 0 involved participants completing the International Physical Activity Questionnaire (IPAQ), menopausal self-questionnaire, collection of anthropometric variables, and an electrocardiogram (ECG) to assess eligibility. Once eligibility was ascertained, those participants were then assigned a flash continuous glucose monitoring (CGM) device and applied on the participant's arm. The screening visit ended with a Chester Step Test to estimate cardiorespiratory fitness (CRF).

At week 1 and 8, participants returned to the laboratory for baseline and post-intervention measures respectively. Both visits involved anthropometric measurements, dual energy X-ray absorptiometry (DEXA) and a 10mL fasting blood sample. Participants also consumed a standardised meal, followed by performing a session of supervised, equipment-free, high-intensity interval exercise. Post-prandial blood capillary blood samples were obtained prior to the exercise (0-EX), immediately after exercise (EX), and 30-minutes post-exercise (30-EX). A schematic depicting the visits is shown in Figure 3.1.

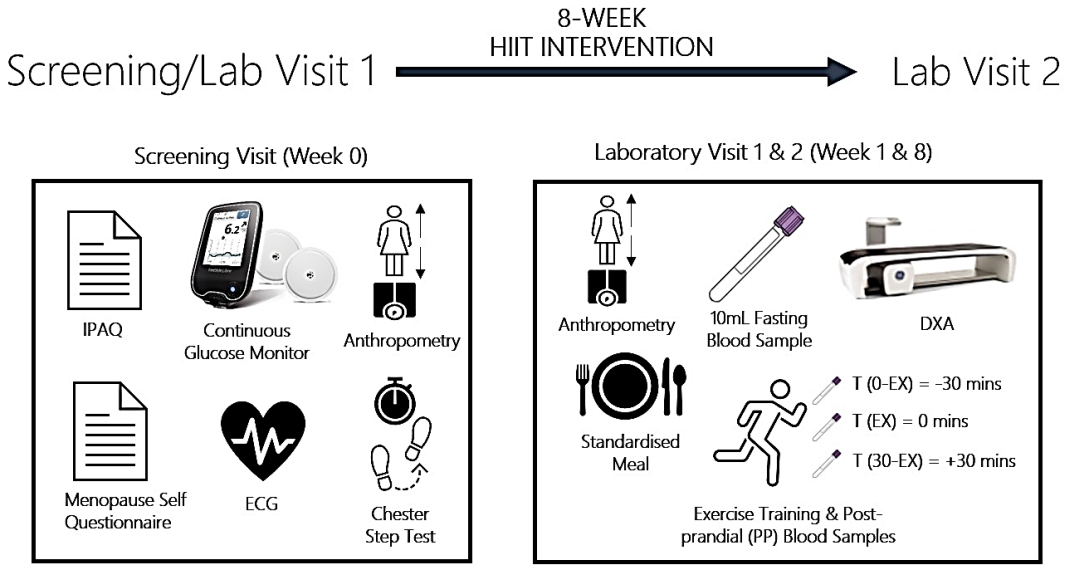

Figure 3.1 Overview of study visit procedures for Chapter 5. IPAQ: International Physical Activity Questionnaire; ECG: electrocardiogram; DEXA: dual energy X-ray absorptiometry.

3.3.2 Overview of study visit procedures: Chapter 6

Following giving informed consent, the screening visit at week 0 involved volunteers completing an IPAQ, menopausal self-questionnaire, anthropometrics, BP and an ECG to assess eligibility. Once eligibility was ascertained, those participants had bio-electrical impedance analysis (BIA) conducted to assess body composition and were randomly allocated into either three groups: control (CTL), exercise only (EX), and exercise and diet (EX+D). The screening visit ended with a Chester Step Test to estimate CRF.

At week 1 and 8, participants returned to the laboratory for baseline and post-intervention measures respectively. Both visits involved anthropometry measurements, BP, BIA, DEXA scan, Chester step test, completion of IPAQ and a 16mL fasting blood sample. Participants allocated to EX+D also completed a Mediterranean diet score sheet in both visits. A schematic depicting the visits is shown in Figure 3.2.

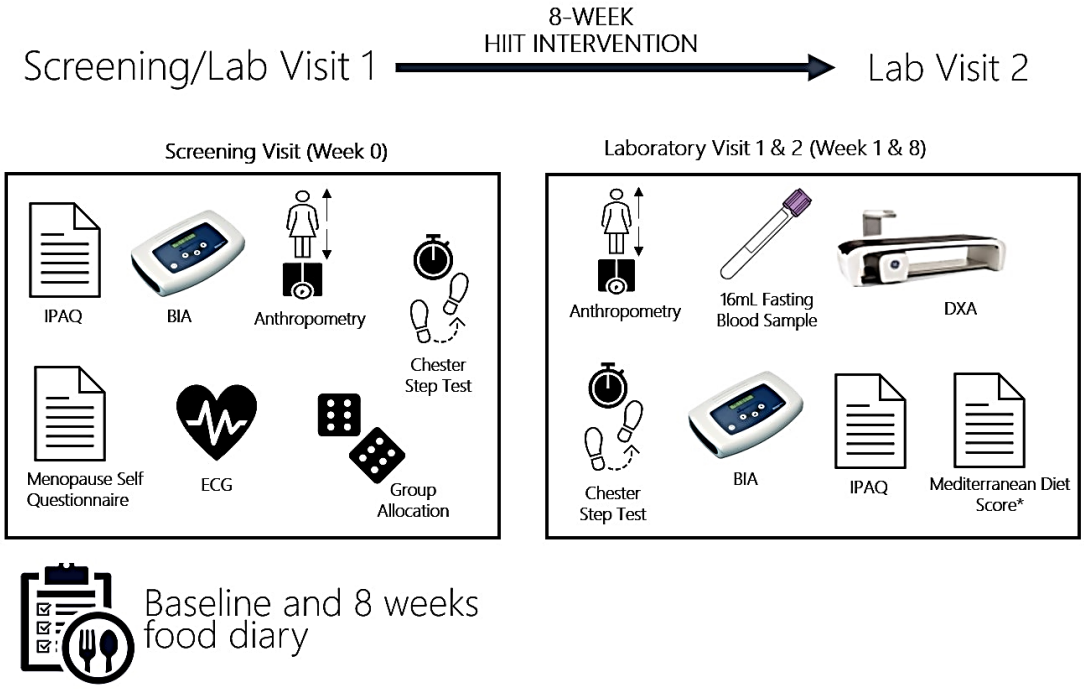

Figure 3.2 Overview of study visit procedures for Chapter 6. IPAQ: International Physical Activity Questionnaire; BIA: bioelectrical impedance analysis; ECG: electrocardiogram; DEXA: dual energy X-ray absorptiometry. *depicts completion by participants in EX+D only.

3.4 Study Visit Procedures

3.4.1 Questionnaires

To ascertain participants' menopausal status and average weekly self-reported physical activity prior to the study, participants completed a menopause self-questionnaire and the International Physical Activity Questionnaire (IPAQ).

International Physical Activity Questionnaire (long version): Prior to acceptance of potential participants to the study, participant's physical activity levels were assessed utilising long IPAQ (Craig et al., 2003) during screening. This 27-item questionnaire involves the evaluation of an array of physical activities including walking, domestic and gardening activities, leisure time, work and transport-related activity. In addition, participants were required to think about the intensity (moderate or vigorous), frequency (measured in days per week), duration (measured in hours and minutes per day) for each specific activity. By weighting each type of activity by its energy requirements defined by metabolic equivalent (METs), a computational score was summated to ascertain the overall level of physical activities:

Table 3.1 Computation of MET-minutes from IPAQ. Participants were deemed eligible for the study if they fell within the "Inactive category" and/or total sum of < 600 METmins (Craig et al., 2003).

Activity	MET	MET-minutes/week
Walking	3.3	3.3*walking minutes*walking days
Moderate	4.0	4.0*moderate-intensity activity minutes*moderate days
Vigorous	8.0	8.0*vigorous-intensity activity minutes*vigorous days
Total MET-minutes		Walking + Moderate + Vigorous MET-min/week

Menopause Self-Questionnaire: Adapted from John Hopkin's Medicine (John Hopkins Medicine, n.d.), participants completed a menopause self-questionnaire for confirmation of post-menopausal status prior to acceptance to the study. This menopause self-questionnaire contained 5 questions relating to menopause status, including the time period prior to menopause and use of hormone replacement therapy (HRT). Menopause denoted the cessation of the menstrual cycle for 12 months, and post-menopause denoted the time after menopause. Participants fell within the eligibility criteria if they were post-menopausal.

3.4.2 Anthropometry and Body Measurements

Anthropometric and body measurements were collected in all study visits for both studies. All measurements were taken by the same researcher to ensure accuracy and repeatability. The addition of the bioelectrical impedance analysis (BIA) machine was utilised in Chapter 6.

Height and weight: Prior to having their anthropometrics measured, participants were instructed to void their bladder and wear light clothing with shoes removed. Height was measured to the

nearest 0.1 cm using a stadiometer (Seca, Hamburg, Germany) and weight was measured to the nearest 0.1 kg using electronic scales (Seca, Hamburg, Germany). BMI was calculated from obtained height and weight.

$$BMI\ (kg/m^2) = \frac{weight\ (kg)}{[height\ (m)]^2}$$

Waist and hip circumference: For measurements of the waist and hip circumference (WC; HC), participants stood with legs parallel and at shoulder-width length. WC was measured against the skin at the narrowest circumference of the torso (above the umbilicus and below the xiphoid process) at the end of normal expiration. For participants that do not have a distinct minimal waist, the umbilical waist circumference (horizontal at the umbilicus) was used (Willis et al., 2007). HC was measured at the maximum circumference around the buttocks. Both WC and HC were measured thrice with the average taken as the final measurement. Waist to hip ratio (W:H) was calculated by dividing HC by WC.

Bioelectrical impedance analysis: The BIA is a low-cost, effective and non-invasive technique to measure body composition. The BIA machine operates by passing a small, imperceptible electrical current through the body and measuring the resistance of the tissues to the flow of the current. The electrical current is generated by the machine and is transmitted through the body via electrodes placed on the skin. The electrical current passes more easily through tissues that contain high water and electrolyte content, such as muscle, than through fat or bone. The BIA machine measures the resistance of the body tissues to the electrical current, which can be used to estimate the amount of body fat, lean mass, and fluid volume in the body (Kyle et al., 2004).

Prior to the procedure, the participant removed their shoes and their right sock, any metal jewellery or metal-containing clothing, and rested for at least five minutes in a supine position. The BIA (BodyStat 1500, BodyStat, Isle of Man, UK) was used to estimate lean mass, body fat mass and body fat percentage. Two electrodes each were placed on the right hand and right foot (one on the protruding bones of the wrist/ankle, one behind the knuckle of the middle finger/toe). Finally, an alligator clip was attached to each end of the electrodes, with the red nearest to the fingers/toes and the black nearest to the wrist/ankle. Measurements of body fat percentage, body fat mass (kg) and lean mass (kg) were recorded.

Dual Energy X-ray absorptiometry: Using two different energy photon beams at 40 keV and 65 keV respectively to differentiate the absorption of bone and soft tissue, the dual-energy X-ray absorptiometry (DEXA) is a non-invasive, low radiation, diagnostic imaging technique that measures whole body bone mass and soft tissues composition. The amount of ionising radiation exposure from one DEXA scan is 0.001mSv. The total dosage of ionising radiation per participant in this study dose is less than 0.005mSv (<5uSv). This is equivalent to less than 1 day of average natural background radiation in the UK. Ionising radiation can cause cancer which manifests itself after many years or decades. Additionally, the risk of developing cancer from taking part in this

study was estimated as 0.000025%. Quality control and maintenance of the machine were routinely carried out by qualified members of staff.

The DEXA (Stratos dR, Diagnostic Medical Systems (DMS) Group, France) was used to measure body composition including total body fat percentage, lean tissue mass, visceral and subcutaneous adiposity. DEXA scans were performed in the first and final lab visits at weeks 1 and 8 respectively using Stratos dR 2D fan-beam scanner located in the A-STEM Research Centre of Swansea University. All scans were performed in a fasted state (at least 12 hours). Prior to the scans, participants were asked to change into lightweight clothing and to void their bladder to minimise influence of hydration status on the results. Any metal jewellery or metal-containing clothing were removed prior to analysis. During the procedure, participants laid in a supine position on the scanner bed, with the scanner arm passing over the body. Participants were positioned as recommended by the manufacturer's instructions, with feet secured in a 20° internal angle foot-positioning tool (Figure 3.3).

A detector located beneath the bed records the levels of X-rays that has passed through the body of which sends this information to a computer that analyses the data and calculates the distribution of body composition. All DEXA images were assessed and corrected to modify the placement of region of interest (ROI) outlines for accurate analysis. The scanner software (Stratos dR version) automatically calculates whole body composition and android ROI. Data were exported into Microsoft Excel and further statistically analysed via SPSS.

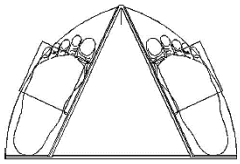

Figure 3.3 Positioning of feet in a 20° internal angle foot-positioning tool (Adapted from Stratos DR (DMS, 2010))

3.4.3 Blood Pressure

Participants were in a rested, seated position or in a supine position (immediately after the DXA scan) for at least ten minutes prior to having their BP taken during the screening visit and laboratory visits respectively. During the BP measurements, the cuff was placed on the supported upper arm with the midline of the bladder placed over the brachial artery of the non-dominant arm. Brachial systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at least five minutes apart, using a digital sphygmomanometer (OMRON M2, OMRON Healthcare, UK). The average of the readings was used as the final measurement.

3.4.4 Cardiorespiratory Fitness

Cardiorespiratory fitness (CRF) is an important health indicator due to its direct association with cardiovascular disease (CVD) risk (Gray et al., 2015). This maximal aerobic capacity (VO_{2max}) is

commonly assessed directly or indirectly via obtaining through an incremental, submaximal exercise test to volitional exhaustion. Undoubtedly, the “gold standard” for assessing cardiorespiratory fitness is measured via cardiopulmonary exercise test (CPET) (Physicians, 2003). However, such procedure involves strenuous exercise which would have deemed participants unsuitable for eligibility in terms of physical activity status for the study (see section 3.4.1). The Chester Step Test, an alternative method for estimating CRF, have shown to serve as a reliable alternative on a test-retest basis without performing strenuous exercise (Bennett et al., 2016).

The Chester Step Test is an incremental, sub-maximal, multi-stage test, devised to reliably measure CRF. Participant’s physical activity levels determine the choice of height used for testing (15cm, 20cm or 25cm), of which participants are required to step on and off the single step to a metronome beat directed by a pre-recorded digital software or audio player. The height of 15cm step was chosen due to its suitability for those over 40 years of age who perform little or no regular physical activity (Sykes, 2018). Comprising of a total of five stages, each stage lasts for two minutes with the maximum test duration of 10 minutes. Stepping commences at 15 steps/minute for 2 minutes during the first stage. At the end of each stage, heart rate (HR) and Borg rate of perceived exertion (RPE; Table 3.2) (Borg, 1982) was recorded. Step rate increases by five more steps/minute at each incremental level and the test continues this incremental pattern until the participant has either reached an RPE of ≥14, 80% of predicted HR_{max} (220 – age), or completed the test. By plotting the exercise HRs attained from each stage on a graphical sheet, a visual line of best fit is then drawn, followed by a horizontal line of the participant’s HR_{max}. VO_{2max} is predicted by matching the oxygen uptake value through the intersection between both lines.

3.4.5 Rate of Perceived Exertion

The Rate of Perceived Exertion (RPE) is a scale from 6 – 20, devised by Gunnar Borg (Borg, 1982) to measure an individual’s effort and exertion during physical work. 6 represent “no exertion at all” and 20 represent “maximal exertion” (Table 3.2). In addition to being a simple and inexpensive tool for exercise prescription and self-regulation, RPE is associated with exercise intensity-related markers including oxygen consumption (VO_2), heart rate and blood lactate (Ciolac et al., 2015). Following each training session, participants were asked to message the researcher their RPE using the 6 – 20 Borg scale. When providing their RPE scores, participants were asked to reflect on the high-intensity intervals during the training session.

Table 3.2 Borg Rate of Perceived Exertion scale *(adapted from Gunnar Borg* (Borg, 1982)*.*

Score	Level of exertion
6	No exertion at al
7	
7.5	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

3.5 Equipment-Free, High-Intensity Interval Training Protocol

Both studies utilised the same equipment-free, high-intensity interval training (EFHIIT) protocol. The home-based EFHIIT (HEFHIIT) program required participants to perform unsupervised HEFHIIT twenty-minutes, thrice a week for 8 weeks (total commitment: 24 sessions). Each session was structure to ensure participants performed at an intensity of ≥80% of maximum heart rate and corresponding to ≥17 Borg rated RPE scale. Participants could choose between two protocols based on their preference and familiarity with EFHIIT:

1. **Progressive Protocol (appendix 1 and 3):**
    - **Weeks 1 – 4:** Participants followed a progressive protocol where the low-intensity intervals decreased progressively. For example:
    - **Week 1:** 60 seconds high-intensity intervals followed by 4 minutes low-intensity intervals.
    - **Week 2:** 60 seconds high-intensity intervals followed by 2.5 minutes low-intensity intervals.
    - **Week 3 – 4:** 60 seconds high-intensity intervals followed by 1.5 minutes low-intensity intervals.
    - **Weeks 5-8:** Participants were encouraged to maintain the interval structure from Week 4 and complete as many repetitions as possible within each 60-second high-intensity interval.

2. **YouTube-based Protocol:**

Participants chose from a selection of EFHIIT protocols available on YouTube, ensuring that the videos met the following criteria:

- The title included "HIIT".
- The exercises were performed without any equipment.
- For video durations that last shorter or longer than 20 minutes, participants should ensure to repeat the video until 20 minutes have been completed or pause at the end of an exercise at the end of 20 minutes.

Examples of suitable YouTube channels included The Body Coach TV, Natacha Océane, FitnessBlender and Juice & Toya. Participants also documented the specific videos used, which included details such as video title, duration, and frequency and notified the researcher at the end of each session (see section 3.5.1).

The exercise modes within the EFHIIT protocols typically included a mix of high-intensity bodyweight exercises such as (appendix 1 and 3):

- Burpees: Combining a squat, push-up, and jump in one fluid movement.

- Jumping Jacks: A cardiovascular exercise that involves jumping to a position with the legs spread wide and the hands touching overhead.
- Mountain Climbers: A bodyweight floor exercise mimicking climbing, performed in a plank position with alternating knee drives.
- High Knees: Running in place, lifting the knees to hip height.

Repetitions and intensity variations were self-regulated to ensure participants reached the target heart rate and RPE. Additionally, participants were encouraged to monitor their performance and adjust as needed to maintain the desired intensity throughout the program. By providing these specific details, the exercise intervention can be more easily replicated, allowing for consistency and comparability in future studies or practical applications.

3.5.1 Recording of adherence and fidelity of intervention

Eligible participants were provided with a Garmin Forerunner 35 accelerometer (Garmin Ltd, United States) throughout the study and were asked to wear the watch on their wrist from the day of the screening visit until the final lab visit (total nine weeks). At the end of each exercise session performed at home, participants were asked to send the details of the session completed to the researcher: 1) average RPE score of the HIIT intervals; 2) the interval durations of the high and low intensities performed. At the end of each participants intervention, accelerometery data was extracted using Garmin software (Garmin Connect, Garmin Ltd, United States) and into Microsoft Excel for further analysis.

3.6 Blood sampling

Prior to all blood sampling, the site of puncture was cleaned thoroughly with alcohol swab. Resting fasting blood samples were drawn from the antecubital vein using venepuncture technique (VACUETTE®, Greiner Bio-One Ltd, Gloucestershire, UK) into 5mL dipotassium ethylenediaminetetraacetic acid (K_2EDTA) Vacutainer™ tubes (BD Vacutainers, Becton Dickinson, New Jersey, USA). All blood tubes collected were inverted five times immediately after collection and were centrifuged at 5000 rpm for 5 minutes within 15 minutes of collection (with the exception of HbA1C, see section 3.7.4). Plasma supernatant obtained were subsequently aliquoted and stored at -80°C for future analysis.

3.6.1 Procedures for Ghrelin Assays

In Chapter 5, post-prandial capillary blood samples were obtained from the fingertip at timepoints 0-EX, EX and 30-EX (pre-exercise, immediately post-exercise and thirty-minutes post-exercise, respectively). A disposable safety lancet (Accu-Chek® Safe-T-Pro Plus Lancets, Roche Diabetes Care Ltd, West Sussex, UK) was used to puncture the skin. To prevent contamination, the first drop of blood was wiped prior to collection. At every timepoint, 200 µL of blood were each collected into two 500 µL K_2EDTA Microvette® (Sarstedt Inc, Nümbrecht, Germany) containing 4-

benzenesulfonyl fluoride hydrochloride (AEBSF) (Sigma-Aldrich, St Louis, Missouri, USA) at a final concentration of 2 mg/mL.

In Chapter 6, 1992 µL of whole blood was immediately aliquoted into a 2 mL collection tube containing 8 µL 4-benzenesulfonyl fluoride hydrochloride (AEBSF) (Sigma-Aldrich, St Louis, Missouri, USA) to achieve a final concentration of 0.4 mg/mL.

3.7 Biochemical Analysis

3.7.1 Enzyme-Linked Immunosorbent Assay

Commercial human-specific Enzyme-Linked Immunosorbent Assay (ELISA) kits were used to determine concentrations of insulin and ghrelin in both studies. In Chapter 5, acyl ghrelin (AG) and total ghrelin (TG) were measured. In Chapter 6, AG, desacyl ghrelin (DAG), leptin and adiponectin were analysed. The specifics of the kits including sensitivity and intraassay coefficient of variations are provided in the respective chapters. Each kit was brought to room temperature before use, and reagents prepared according to manufactures instructions. All plasma samples were thawed and centrifuged for 4000 rpm for 5 minutes prior to assaying.

ELISA is a widely used laboratory technique for detecting and quantifying the presence of specific proteins or antibodies in biological samples. The ELISA method involves immobilising a specific antigen or antibody onto a solid surface, such as a microplate, and then adding the sample containing the target protein or antibody. The target protein or antibody binds to the immobilised antigen or antibody, and any unbound proteins or antibodies are washed away. A secondary antibody, tagged with an enzyme such as horseradish peroxidase or alkaline phosphatase, is then added, which binds specifically to the target protein or antibody. After washing away the unbound secondary antibody, a substrate for the enzyme is added, causing a detectable signal to be produced, which is proportional to the amount of the target protein or antibody present in the sample. ELISA is a highly sensitive and specific method and can be used for a wide range of applications, including disease diagnosis, monitoring of therapeutic interventions, and basic research. All ELISA kits used the sandwich technique and underwent the colorimetric reaction.

3.7.1.1 Chapter 5: ELISA

Plasma obtained from participants previously described in section 3.6.1 were used for AG and TG quantification. AG and TG were analysed using ELISA assays (EZGRA-88K and EZGRT-89K respectively) purchased from Sigma-Aldrich. For both protocols, 20 µL of matrix solution was added to each well, followed by 10 µL/30 µL depending on the well. Subsequently, 20 µL of standards, quality control (QC) and samples were added into the respective wells. The addition of a 50 µL capture antibody mixture was followed by a 2-hour incubation at room temperature. After washing/aspiration of plates, 100 µL of enzyme solution was added per well. Following a 30-minute incubation, the plates were washed again before introducing 100 µL of substrate. The plate underwent a 15-minute incubation in the dark for colour development, which was stopped by the addition of 100 µL of stop solution. Absorbance readings were taken at 450 nm and 590

nm using a plate reader, where AG and TG concentrations were determined by comparing unknown samples to the standard curve.

3.7.1.2 Chapter 6: ELISA

Acyl and des-acyl ghrelin: Plasma obtained from participants previously described in section 3.6.1 were used for AG and DAG quantification. AG and DAG were measured using assays purchased from Bertin Bioreagent (Cat# A05306 and Cat# A05306 respectively). Prior to the assays, plasma samples were diluted in EIA buffer provided by the kit at dilutions of 1:2 for AG, and 1:5 for DAG.

Each plate was washed five times with wash buffer prior to usage, following by 100 µL of EIA buffer dispensed to non-specific binding (NSB) wells. Next, 100 µL of standard, QC and samples were dispensed into the respective wells, followed by incubation for 2 hours. The plates were washed and aspirated, followed by 100 µL of acylated ghrelin tracer/unacylated ghrelin tracer per well for AG and DAG respectively. The plates were incubated again for 2 hours and underwent wash/aspiration. The addition of 200 µL of Ellman's reagent was added and incubated for at least 30 minutes in the dark for optimal colour development. At wavelength between 405 nm and 414 nm, AG and DAG concentrations were determined via comparison of unknown samples against the standard curve.

A*diponectin and Leptin:* Adiponectin and leptin were measured using DuoSet ELISA purchased from R&D (Cat# DY1065 and Cat# DY398 respectively). Prior to the assays, the plates were prepared at least 24 hours prior to usage. Each plate was coated with 100 µL of diluted capture antibody, sealed and incubated overnight.

Following overnight incubation, plates were washed with wash buffer, blocked with 300 µL of reagent diluent and subsequently incubated for 1 hour. Following wash/aspiration, 100 µL of standard and sample were added to respective wells. The plates were incubated for 2 hours followed by wash/aspiration. Next, 100 µL of detection antibody was added and incubated for 2 hours. The plates were washed/aspirated, followed by 100 µL of Streptavidin-HRP added to each well. The plates were incubated for 20 minutes away from direct light. Following a final wash, 100 µL of substrate solution were added to each well. The plates were incubated for another 20 minutes away from direct light before the addition of 50 µL of stop solution. Absorbance was determined with wavelengths at 450nm and 540nm, where final readings were calculated by subtracting readings at 540 nm from 450 nm. Concentrations of adiponectin and leptin were determined via comparison of unknown samples against the standard curve.

3.7.2 Insulin Chemiluminescence Assay

Insulin levels were determined for both Chapters 5 and 6 using immunometric assay purchased from Invitron (IV2-001). This assay uses molecular light technology chemiluminescence to quantify measurements of insulin by employing insulin-specific solid phase antibody immobilised on the wells, and a soluble antibody labelled with chemiluminescent acridinium ester. The labelled antibody solution is then incubated with the plasma sample, which is then subsequently washed

to remove unbound labelled antibody prior to measurement. The bound luminescence is quantified by a luminometer to quantify insulin concentrations. The insulin kit was brought to room temperature before use, and reagents prepared according to manufactures instructions. All plasma samples were thawed and centrifuged for 4000 rpm for 5 minutes prior to assaying.

The protocol required 100 µL of labelled antibody solution, followed by 25 µL of standard, control and samples to respective wells. The plate was incubated for 2 hours, followed by washing/aspiration. The absorbance was read with a luminometer where insulin concentrations were determined via comparison of unknown samples against a four-point cubic spline curve.

3.7.3 Randox Daytona Plus

Plasma aliquoted from fasting venepuncture blood samples collected from in both studies were analysed using the Randox Daytona Plus. This is a clinical laboratory system that utilises immunoturbidimetry and photometry methods to measure several essential blood components in serum and plasma rapidly. The instrument is widely used to analyse the concentrations of glucose, cholesterol, HDL, albumin, creatinine, and triglycerides in patient samples. To ensure accurate measurements, the samples are processed alongside quality control samples and calibrated using Calibration Serum Level 3 and saline. Using direct photometry, the instrument detects a coloured endpoint, and the concentrations of the blood components are measured in mmol/L. To calculate the LDL concentration of the samples, the Friedewald equation was employed to the values produced by the Randox Daytona Plus:

$$LDL\ (mmol/L) = Total\ Cholesterol\ (mmol/L) - HDL\ (mmol/L) - \frac{Triglycerides\ (mmol/L)}{2.2}$$

3.7.4 Glycated Haemoglobin

Fasting whole blood collected in K_2EDTA vacutainers from venepuncture were used to analyse HbA1c using the Tosoh G8 high-performance liquid chromatography (HPLC) analyser (Tosoh Biosciences, Inc.; USA). HbA1c (glycated haemoglobin) is a type of haemoglobin that forms when glucose molecules in the blood bind to haemoglobin molecules. This process occurs over several weeks, and the level of HbA1c reflects the average blood glucose levels over that time. HbA1c is a widely used measure for monitoring and diagnosing diabetes, as it provides an accurate reflection of long-term blood glucose control. HbA1c levels are expressed as a percentage of the total haemoglobin in the blood and are used to assess the effectiveness of diabetes treatment and to determine the risk of complications associated with high blood sugar levels.

The Tosoh HPLC analyser is a sophisticated system widely used in clinical laboratories to measure and quantify haemoglobin variants and glycohemoglobin (HbA1c) levels in blood samples. To analyse HbA1c, 5 µL of venous whole blood sample were pre-diluted in 1500 µL of buffer. Utilising the HPLC method, haemoglobin components are separated based on their unique electrical charges and physical characteristics. By measuring the differences in retention times and peak areas of these components, the Tosoh HPLC analyser can accurately determine the

types and amounts of haemoglobin present in a sample. This system is highly sensitive and precise, allowing for the accurate identification of variant haemoglobins that may cause haematological disorders.

3.8 Statistical Analysis

All statistical analysis of raw data were performed using SPSS (IBM SPSS Statistics, version 22.0) and Microsoft Excel. Statistical significance was set at p-value of <0.05 for all tests and data expressed as mean ± standard deviation (SD) unless stated otherwise.

3.8.1 Insulin sensitivity analysis

Using data from FBG and fasting insulin levels, insulin sensitivity analysis was calculated using the homeostasis model assessment 2 (HOMA2) Calculator version 2.2 (University of Oxford, Oxford, UK). HOMA is a calculator tool formulated in 1985 that accounts FBG, fasting insulin and/or C-peptide to measure insulin sensitivity (HOMA-%S), insulin resistance (HOMA-IR), and beta-cell function (HOMA-%B (Matthews et al., 1985). Updated in 1996, HOMA2 emerged with better predictions of diabetes progression evaluating HOMA2-%S, HOMA2-IR and HOMA2-%B (Song et al., 2016).

3.8.2 Continuous Data

Data was checked for normality using Shapiro-Wilk. If normally distributed, continuous data is summarised by mean ± SD, and by median and interquartile range (IQR) if not normally distributed. Comparisons for within-subjects data were performed using the student paired t-test for parametric variables, or Wilcoxon signed-rank test for non-parametric variables. Comparisons for within-group variables, one-way analysis of variance (ANOVA), repeated-measures ANOVA with Bonferroni corrections was used to compare the mean for normally distributed data. Alternatively, non-normally distributed data was analysed using Kruskal-Wallis test. For the analysis of association between two continuous variables within normally distributed data, a Pearson correlation coefficient test was conducted, within non-normally distributed data, a Spearman's Rho test was conducted.

Chapter 4 - Effects of exercise training on cardiometabolic risk factors in post-menopausal women – a systematic review and meta-analysis of randomised controlled trials

4.1 Introduction

Post-menopausal women are at an increased risk of CMD, of which unhealthy lifestyle habits including physical inactivity and sedentarism can exacerbate this risk (Ra & Kim, 2021; Remie et al., 2021). Exercise training has been shown to elicit reductions in independent cardiometabolic risk factors in post-menopausal women through improvements in: SBP and DBP (Xi et al., 2021), inflammatory markers (Khalafi et al., 2021), endothelial function (Jaime et al., 2019), body composition (Kim & Kim, 2012), insulin resistance (IR) (Son & Park, 2021), HDL (Kodama et al., 2007) and cardiorespiratory fitness (CRF) (Arsenault et al., 2009). MetS precedes CMD, as previously described in section 2.1.3. Thus, it is important to explore the effectiveness of exercise training on benefitting MetS risk factors.

4.1.1 Exercise Modalities and Cardiometabolic Benefits

As a non-pharmacological approach, regular physical activity or exercise training of all types has demonstrated efficacy and significance in improving cardiometabolic health in adults with overweight/obesity (Batrakoulis et al., 2022). Yet, the effectiveness of exercise interventions depends largely on the type of exercise employed. This section introduces the exercise modalities of aerobic, resistance, combined and interval training and their cardiometabolic health benefits.

Aerobic training (AT) has been acknowledged to improve metabolic and CVD risk factors in healthy adults (Wang et al., 2022), as well as in those with health conditions including hypertension (de Barcelos et al., 2022), MetS (Lemes et al., 2018) and T2D (Amin et al., 2023). According to the ACSM, aerobic exercise (AE) is defined as any activity and utilises large muscle groups that can be maintained in a rhythmic, continuous nature (American College of Sports Medicine, 2014). This modality is recommended to improve cardiovascular fitness. Examples include walking, running, cycling, swimming and dancing (American College of Sports Medicine, 2014). An alternative to AT, resistance training (RT) is another exercise modality characterised by muscular activities that work against an external load (American College of Sports Medicine, 2014). By stimulating increased muscle protein turnover, RT enhances muscle strength and mass, offering benefits for sarcopenia-related characteristics as well as MetS-related risk factors (Cho et al., 2022). MetS and sarcopenia are interrelated through factors, including adiposity and insulin resistance (IR), with decreased muscle strength and mass associated with MetS development (Nishikawa et al., 2021).

Combined training (CT) of AT and RT have shown superiority than either modality alone. Current health guidelines for the management of obesity in adults recommends regular multimodal exercise of ≥250 mins/week of AT, supplemented with 2 – 3 bouts of RT for clinical meaningful weight loss aimed to improve cardiometabolic health indicators (Ashton et al., 2020). It has been theorised that energy expenditure (EE) during exercise bouts performed during AT is higher than that during RT, leading to more favourable reductions in fat mass (Schwingshackl et al., 2013). On the other hand, multiple meta-analyses reported that RT was superior to AT in reducing SBP and DBP (Liang et al., 2021; Loaiza-Betancur et al., 2021). It has been suggested that RT

mediates reductions in BP by lowering systemic vascular resistance through improved endothelial sensitivity to nitric oxide (NO), a potent vasodilator, resulting reductions in sympathetic tone and salt load (Saladini, 2022). Additionally, findings from a meta-analysis corroborate with latest exercise guidelines, showing that CT is the most effective modality for improving cardiometabolic health-related outcomes in adults with overweight and obesity (Batrakoulis et al., 2022).

Despite its benefits, the large weekly time demand poses potential drawbacks to CT, with lack of time consistently reported as the main barrier to engaging in physical activity (Koh et al., 2022). Interval training, involving alternating periods of high- and low-intensity exercise, has emerged as an efficient and time-effective exercise approach. High-intensity interval training (HIIT) and sprint interval training (SIT) involve short bursts of high-intensity exercise interspaced by recovery periods of low-intensity exercise or rest. HIIT involves intermittent high-intensity exercise performed at 80% to 95% of maximum heart rate (HRmax), while SIT requires ‘all-out’ efforts at supramaximal work rates (MacInnis & Gibala, 2017). Interval training has gained popularity as it appeals to the most commonly cited barrier to physical activity of ‘lack of time’, while providing similar or better cardiometabolic outcomes (Batacan et al., 2017) and fat oxidation (Atakan et al., 2022) compared to moderate-intensity continuous training (MICT).

4.2 Aims

Individuals with MetS have twice the risk of CVD events compared to those without MetS (Mottillo et al., 2010). Furthermore, the prevalence of MetS is strongly associated with age (Hildrum et al., 2007), and this risk is exacerbated in women following the menopausal transition (Torréns et al., 2009). There is limited robust research examining the efficacy of exercise intensity and modality on combined risk factors focused on MetS progression within predisposed post-menopausal women. Although regular physical activity is advised and considered as a non-pharmacological alternative to improve cardiometabolic health, the exercise dosage in terms of intensity, duration, as well as the modality in ameliorating MetS risk factors remains unclear.

This study aims to:

1) Systematically review and meta-analyse randomised controlled trials assessing the effect of exercise training on individual MetS risk factors in post-menopausal women.
2) Determine which exercise intensity, modality and duration have the most beneficial impact on MetS risk factors in post-menopausal women.

Therefore, it is hypothesised that:

1) Exercise training improves MetS risk markers.
2) Differing intensity, modality and duration improves MetS risk markers.

Primary end point:

1) Change in individual MetS risk factors following exercise training compared to control groups.

Secondary end points:

1) Meta-regression of BMI and health status on the MetS outcomes.
2) Change in individual MetS risk factors for differing exercise intensity, modality and duration.

4.3 Materials and Methods

4.3.1 Registration

This review was registered at PROSPERO (registration number CRD42021283944). This systematic review and meta-analysis was performed in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement guidelines and the Cochrane Handbook of Systematic Reviews of Interventions (Liberati et al., 2009).

4.3.2 Eligibility Criteria

The following pre-defined criteria were employed to the study inclusion:

2) Randomised-controlled trials (RCT)
3) Studies explicitly including women who are post-menopausal (defined by at least one year of amenorrhea and/or follicle stimulating hormone (FSH) levels ≥30 IU/L)
4) Peer-reviewed, full-text studies with training program lasting at least 8 weeks, in a pre-post design
5) Studies analysed and reporting the effects of exercise training in at least one variable of MetS (fasting blood glucose (FBG), high-density lipoprotein (HDL), triglycerides (TRG), SBP, DBP, and/or waist circumference (WC)
6) Blood measurements had to be performed in a fasted state categorised as >8 hours without food or after an overnight fast
7) Studies containing an exercise-only arm if the study is a multicomponent treatment. If studies included men or pre-/peri-menopausal women, outcome variables of post-menopausal women had to be analysed separately.

Papers were excluded if:

1) Post-menopausal status was not predefined in the inclusion criteria
2) Women had cancer or non-alcoholic fatty liver disease (NAFLD)
3) Not published in peer-reviewed journals
4) Not written in the English language; 5) conducted in animals
5) Addressing interventions applying novel exercise technologies (e.g., whole-body vibration, exergaming etc.)
6) Not of RCT design, review articles, literature reviews, study protocol, abstracts or conference papers

4.3.3 Search Strategy

All literature investigating the effect of exercise training on risk factors of MetS in post-menopausal women were searched and obtained utilising PubMed, Scopus, web of science and the Cochrane Central Register of Controlled Trials from inception to December 2021. The search strategy included various combinations of the keywords and MeSH terms: “postmenopausal”, “post-menopausal”, “older women”, “exercise training”, “aerobic exercise”, “aerobic training”, “strength training”, “resistance training”, “physical training”, “physical exercise”, “exercise”, “metabolic syndrome”, “cardiometabolic disease”, “cardiometabolic syndrome”, “metabolic health”, “blood pressure”, “fasting glucose”, “glycaemic control”, “glycaemia, “waist circumference”, “anthropometry, “lipid profile”. Boolean search terms (AND, OR) were utilised. A detailed search strategy is presented in the Appendix 2. These searches were limited to RCTs and human studies. Papers accepted were in English language only. To increase generalisability of results, papers were accepted regardless of the participants’ health status (except cancer or NAFLD). In addition, reference lists of all relevant systematic reviews and meta-analysis were searched manually to locate additional relevant studies.

Database results were imported into Covidence systematic review software (Veritas Health Innovation, Australia). Abstracts and titles were independently reviewed by two reviewers (A.T and R.C). Papers were initially classified as ‘yes’, ‘no’ or ‘maybe’, of which those classified as ‘yes’ or ‘maybe’ proceeded to full-text screening. Full-text papers were then classified as ‘yes’ or ‘no’ with subsequent final papers classified as ‘yes’. Any disagreements were resolved by reaching a consensus.

4.3.4 Risk of Bias and Quality Assessment

The revised Cochrane Risk of Bias 2 tool (RoB 2) was independently used by two authors (A.T and R.C) to assess risk of bias. The following aspects were evaluated for the quality of the studies: 1) bias arising from the randomisation process; 2) bias due to deviations from the intended interventions; 3) bias due to missing outcome data; 4) bias in the measurement of the outcome; 5) bias in the selection of the reported result. The details of the RoB2 assessment are provided in the Appendix 2. The overall risk of bias for each study was determined as low risk, some concerns, or high risk. Any disagreements were examined by all authors before reaching a consensus. Sensitivity analyses were conducted by omitting each individual study and evaluating the effect on standardised mean differences (SMD) or mean differences (MD), and heterogeneity.

4.3.5 Data Extraction

Extraction of data from included studies were performed by a single author (A.T) into an electronic spreadsheet (Excel 2016, Microsoft Corporation USA) according to the following study characteristics: (A) first author; (B) year of publication; (C) study design; (D) characteristics of the participants including health status, mean age, baseline body mass index (BMI) and sample size; (E) exercise training characteristics including exercise modality, duration and frequency; (F) pre-

and post-intervention measurements of MetS outcome variables (FBG, HDL, TRG, SBP, DBP and/or WC) and corresponding measurements of MetS outcome variables (FBG, HDL, TRG, SBP, DBP and/or WC) in the non-exercise control group. If studies had multi-interventions arms, only data of exercise and control (non-exercise) arms were included. All data extracted were checked for accuracy by a second author (R.C).

Following data extraction, FBG, HDL, TRG, SBP and DBP were converted to SI units (BG, HDL and TRG: mmol/L). For each of the six outcomes of interest, mean change scores were calculated from pre- and post-intervention mean and standard deviation (SD) values in both the exercise and control arms for the meta-analyses. In studies reporting 95% confidence intervals, interquartile range (IQR) or standard error (SE), these were converted to a standard deviation by these equations respectively:

$$SD = \left(\sqrt{n}\right) \times \frac{(upper\ limit - lower\ limit)}{2 \times T.INV(0.05; n-1)}; SD = IQR\ /\ 1.35;\ SD = SE \times \left(\sqrt{n}\right)$$

n is the sample size, and T.INV represents the function specific to the t-distribution (Higgins et al., 2011). Additionally, WebPlotDigitizer Version 4.2 (Ankit Rohatgi, USA) was used for the extraction of data from graphs and figures when required. One study was excluded as no response was received when the corresponding author was contacted due to insufficient data (Adams-Campbell et al., 2021).

4.3.6 Data Synthesis and Analysis

Data synthesis and analysis were performed by one author (A.T), statistical analyses were completed utilising JASP (JASP Software version 0.16.4, JASP, Amsterdam, Netherlands) and Review Manager software (RevMan Version 5.4, Cochrane Collaboration, Oxford, UK). Using the random-effects model, SMD or MD with 95% confidence (CI) were calculated. Heterogeneity was assessed utilising the I^2 statistic, with >50% indicating large heterogeneity. To establish the magnitude of the effects of exercise training vs control on all MetS risk factors, effect sizes were calculated in accordance with Cochrane guidelines using the following: 0.2 – 0.49, 0.5 – 0.79 and ≥ 0.8 for small, moderate and large effects respectively (Cohen, 2013).

Six separate pooled meta-analyses were conducted for each of the MetS risk factors. Sub-group analyses of exercise intensity were performed for all MetS risk factors in accordance with Table 4.1. Studies that included a combination of intensities used for exercise training were denoted as light-moderate, light-vigorous, and moderate-vigorous. Similarly, exercise modality (continuous, resistance, combined or interval (defined by A.T)) and intervention duration (short term: <12 weeks; long term: ≥12 weeks; very long term: ≥6 months) were included.

Meta-regressions were also performed to determine the potential effect of participant characteristics on all MetS risk factors: continuous covariate (BMI) and categorical covariate (health status). Publication bias of included studies for all MetS risk variables were assessed

using visual interpretation of funnel plots. Egger's regression test of $p < 0.05$ was used as a secondary determinant to confirm significant publication bias (Egger et al., 1997).

Table 4.1 Criteria for exercise intensity classification in accordance with The American College of Sports Medicine guidelines (American College of Sports Medicine, 2014)***.*** METs: Metabolic Equivalents; RPE: Rate of Perceived Exertion; RM: Resistance Maximum.

	Very light	**Light**	**Moderate**	**Vigorous**	**Very vigorous**
Oxygen uptake (VO_2max) (%)	< 20	20 – 39	40 – 59	60 – 84	≥ 85
Heart rate reserve (HRR%)	< 20	20 – 39	40 – 59	60 – 84	≥ 85
Maximum heart rate (%)	< 50	50 – 63	64 – 76	77 – 93	≥ 94
Metabolic Equivalent of Task (METs) (MET Unit)		< 3	3 – 6	> 6	
RPE (Borg scale unit)	≤ 10	10 – 12	13 – 14	15 – 16	17 - 18
1-RM (%)		≤ 50	60 – 70	> 70	> 100

4.4 Results

4.4.1 Study Selection

A total of 5,452 papers were initially identified from database searches. After removal of 888 duplicates, title and abstract screening excluded 4,413 studies. 151 papers were sought for retrieval for full-text versions, of which 7 were removed due to no full-text available. Of the remaining 144 full-text papers retrieved, 105 were excluded (28 had no MetS variables analysed, 16 had inappropriate study design, 12 had duplicated data, 15 reported inadequate outcomes, 2 did not analyse post-menopausal women separately, 2 were non-English language, 28 did not predefine post-menopause status in their inclusion criteria and 2 did not state that blood measurements were taken in a fasted state/following an overnight fast). 39 final papers were identified to be eligible for inclusion in the review and meta-analysis (Akwa et al., 2017; Azadpour et al., 2017; Bergström et al., 2009; Biteli et al., 2021; Chagas et al., 2017; Church et al., 2007; Colado et al., 2009; Conceição et al., 2013; Dalleck et al., 2009; Figueroa et al., 2011; Frank et al., 2005; Friedenreich et al., 2011; Gómez-Tomás et al., 2018; Hettchen et al., 2021; Jaime et al., 2019; Keyhani et al., 2020; Kim & Kim, 2012; Latosik et al., 2014; Lee et al., 2012; Lee et al., 2021; Lesser et al., 2016; Libardi et al., 2012; Marcus et al., 2009; Miyaki et al., 2012; Moreau et al., 2001; Neves et al., 2017; Nunes et al., 2016; Rezende Barbosa et al., 2019; Sénéchal et al., 2012; Seo et al., 2010; Son et al., 2017; Son & Park, 2021; Staffileno et al., 2001; Trabka et al., 2014; Van Gemert et al., 2015; Ward et al., 2020; Wong et al., 2018, 2019; Wooten et al., 2011). 1 paper (Biteli et al., 2021) conducted multiple studies of the same intervention in two different cohorts of interest each, a total of 40 separate studies were included in the analysis. The PRISMA diagram of the selection process is detailed in Figure 4.1.

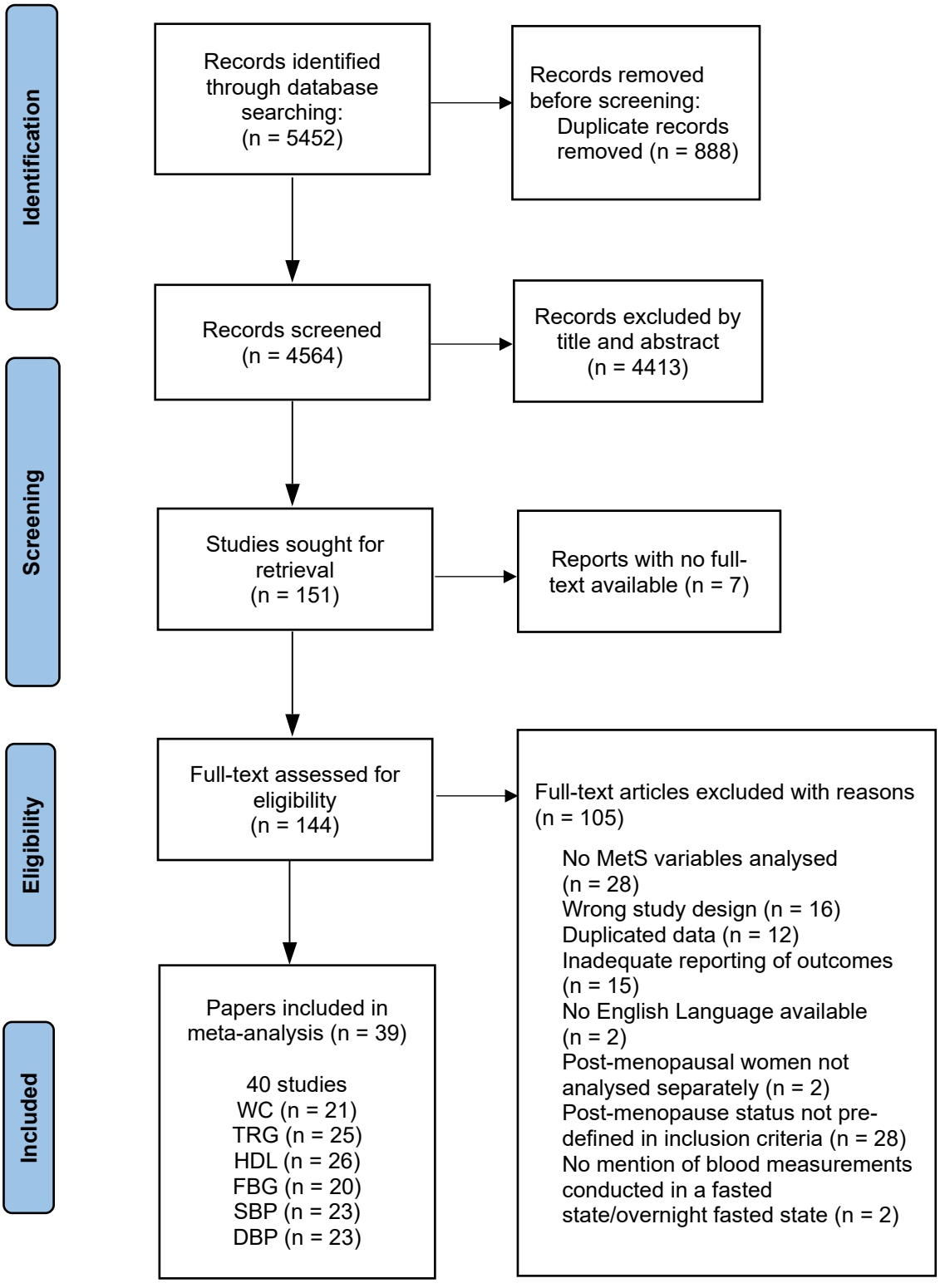

Figure 4.1 PRISMA flow diagram of the study selection process. WC: waist circumference; TRG: triglycerides; HDL: high-density lipoprotein; FBG: fasting blood glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure.

4.4.2 Study Characteristics

Characteristics of the exercise interventions and participants are described in Table 4.2. The mean ± SD age and BMI of the participants in the studies ranged from 52.9 ± 1.9 years (Colado et al., 2009) to 76.0 ± 5.0 years (Son et al., 2017), and 22.2 ± 2.0 kg/m^2 (Miyaki et al., 2012) to 34.0 ± 1.3 kg/m^2 (Wooten et al., 2011), respectively. All participants were post-menopausal (defined by at least one year of amenorrhea and/or follicle stimulating hormone (FSH) levels ≥30 IU/L). Many of the studies were performed in overweight or obese individuals with no additional MetS risk factors (27 studies) (Akwa et al., 2017; Bergström et al., 2009; Biteli et al., 2021; Chagas et al., 2017; Church et al., 2007; Colado et al., 2009; Conceição et al., 2013; Dalleck et al., 2009; Frank et al., 2005; Friedenreich et al., 2011; Gómez-Tomás et al., 2018; Keyhani et al., 2020; Kim & Kim, 2012; Lee et al., 2012; Lee et al., 2021; Lesser et al., 2016; Libardi et al., 2012; Marcus et al., 2009; Neves et al., 2017; Nunes et al., 2016; Rezende Barbosa et al., 2019; Seo et al., 2010; Son & Park, 2021; Trabka et al., 2014; Van Gemert et al., 2015; Ward et al., 2020; Wooten et al., 2011). There were a total of 7 studies conducted in women with hypertension (Azadpour et al., 2017; Kim & Kim, 2012; Latosik et al., 2014; Moreau et al., 2001; Son et al., 2017; Wong et al., 2018, 2019), 1 study in women with dyslipidaemia (Biteli et al., 2021), 1 study in women with osteopenia (Hettchen et al., 2021), and 1 study in women with dynapenia (Sénéchal et al., 2012). The remaining 3 studies were in healthy women of normal weight (Figueroa et al., 2011; Jaime et al., 2019; Miyaki et al., 2012).

A total of 2,132 participants were included, with 1,069 and 1,023 participants in the exercise and control groups respectively. Each MetS variable encompassed the following number of studies and total participants: WC: 21 studies, 1,198 participants; TRG: 25 studies, 1,064 participants; HDL: 26 studies, 1,035 participants; FBG: 20 studies, 1,103 participants; SBP: 23 studies, 877 participants; DBP: 23 studies, 877 participants. In 1 study (Biteli et al., 2021), two different cohorts of women (women with or without dyslipidaemia) were analysed separately.

The exercise interventions were diverse amongst the studies. They consisted of a range of intensities classified by Table 4.1. The duration of the interventions ranged from 8 weeks to 12 months. The intensity of the exercise sessions increased periodically over the course of the program, with measurements of heart rate and intensity regularly monitored.

4.4.3 Risk of Bias

The risk of bias for selected studies are provided in the Appendix 2. Overall, 3 studies were reported as low risk, 22 studies as some concerns and 15 studies as high risk of bias. Blinding of participants to their allocation of exercise intervention is not possible in exercise-related studies. Hence, allocation concealment under the domain “bias from randomisation process” was not described in detail in all studies. We therefore evaluated this aspect as “some concerns”. 10 studies reported acceptable method of random sequence generation (i.e. computer generated), whilst the remaining 30 studies were judged as “some concerns” due to insufficient detail reported for randomisation method.

Table 4.2 Summary of characteristics of participants and interventions in 40 studies.

Study (Country)	Participants characteristics	Age (years); BMI (kg/m^2)	No. of Participants	Exercise Intervention			MetS Risk Factors
				Duration	Frequency	Modality (intensity)	
Akwa et al., 2017 (Ghana)	Healthy	EX: 61.3 ± 7.5; 31.2 ± 7.5 CON: 61.3 ± 7.8; 29.0 ± 5.4	EX: 8 CON: 10	8 weeks	3 days	Continuous (light-moderate intensity)	HDL, TRG, SBP, DBP
Azadpour et al., 2017 (Turkey)	Obese with prehypertension	EX: 57.6 ± 4.3; 32.2 ± 1.8 CON: 56.6 ± 4.2; 31.3 ± 1.4	EX: 12 CON: 8	10 weeks	3 days	Continuous (moderate-vigorous intensity)	SBP, DBP, WC
Bergström et al., 2009 (Sweden)	Healthy overweight	EX: 58.5 ± 4.2; 24.2 ± 2.5 CON: 59.4 ± 3.6; 25.0 ± 2.2	EX: 48 CON: 44	12 months	4-5 days	Continuous (moderate intensity)	HDL, SBP, DBP, WC
Biteli et al., 2021[a] (Brazil)	Dyslipidaemic obese	EX: 62.3 ± 6.7; N/A CON: 59.3 ± 6.2; N/A	EX: 24 CON: 22	20 weeks	3 days	Combined (moderate intensity)	FBG, HDL, TRG, WC
Biteli et al., 2021[b] (Brazil)	Obese	EX: 58.5 ± 6.5; N/A CON: 61.2 ± 7.7; N/A	EX: 11 CON: 13	20 weeks	3 days	Combined (moderate intensity)	FBG, HDL, TRG, WC
Chagas et al., 2017 (Brazil)	Healthy obese	EX: 61.3 ± 6.4; 30.6 ± 5.0 CON: 59.8 ± 7.1; 32.8 ± 4.9	EX: 35 CON: 35	20 weeks	3 days	Combined (moderate intensity)	FBG, HDL, TRG, WC
Church et al., 2007 (USA)	Overweight/obese	EX: 56.6 ± 6.6; 31.3 ± 3.6 CON: 57.2 ± 5.8; 32.3 ± 3.9	EX: 103 CON: 102	6 months	3-5 days	Continuous (Moderate intensity)	FBG, HDL, TRG, SBP, DBP, WC
(Colado et al., 2009 (Spain)	Healthy	EX: 54.0 ± 2.8; 29.5 ± 3.3 CON: 52.9 ± 1.9; 27.5 ± 3.3	EX: 21 CON: 10	24 weeks	3 days	Resistance (Moderate intensity)	FBG, HDL, TRG, SBP, DBP, WC
Conceição et al., 2013 (Brazil)	Healthy	EX: 53.4 ± 4.0; 26.2 ± 3.3 CON: 53.0 ± 5.7; 25.3 ± 1.8	EX: 10 CON: 10	16 weeks	3 days	Resistance (moderate-vigorous intensity)	FBG, HDL, TRG, SBP, DBP, WC
Dalleck et al., 2009 (USA)	Healthy	EX: 55.4 ± 3.2; 28.1 ± 4.5 CON: 57.4 ± 4.6; 30.0 ± 8.7	EX: 8 CON: 10	12 weeks	5 days	Continuous (moderate intensity)	FBG, HDL, TRG, SBP, DBP, WC
Figueroa et al., 2011 (Korea)	Healthy	EX: 54.0 ± 2.0; 24.2 ± 0.7 CON: 54.0 ± 1.0; 23.1 ± 0.7	EX: 12 CON:12	12 weeks	3 days	Combined (moderate intensity)	SBP, DBP
Frank et al., 2005 (USA)	Overweight	EX: 60.7 ± 6.7; 30.4 ± 4.1 CON: 60.6 ± 6.8; 30.5 ± 3.7	EX: 87 CON: 86	12 months	5 days	Continuous (moderate intensity)	FBG, TRG
Friedenreich et al., 2011 (Canada)	Healthy	EX: 61.2 ± 5.4; 29.1 ± 4.5 CON: 60.6 ± 5.7; 29.2 ± 4.3	EX: 160 CON: 160	12 months	5 days	Continuous (moderate-vigorous intensity)	WC
Gómez-Tomás et al., 2018 (Spain)	Healthy	EX: 70.9 ± 4.4; 28.7 ± 4.5 CON: 70.5 ± 5.4; 30.2 ± 5.6	EX: 18 CON: 20	12 months	3 days	Resistance (light-moderate intensity)	HDL, TRG, WC

Table 4.2 (continued)

Hettchen et al., 2021 (Germany)	Osteopenic	EX: 53.6 ± 2.0; 23.7 ± 3.4 CON: 54.5 ± 1.6; 24.9 ± 4.8	EX: 27 CON:27	13 months	3 days	Continuous (vigorous intensity)	FBG, HDL, TRG, WC
Jaime et al., 2019 (USA)	Healthy	EX: 64.0 ± 1.0; 24.0 ± 0.6 CON: 67.0 ± 1.0; 22.5 ± 0.9	EX: 21 CON: 14	12 weeks	N/A	Resistance (light intensity)	SBP, DBP
Kim & Kim, 2012 (Korea)	Obese	EX: 53.4 ± 2.4; 25.0 ± 1.3 CON: 54.5 ± 2.8; 25.1 ± 1.5	EX: 15 CON: 15	16 weeks	3 days	Continuous (moderate-vigorous intensity)	FBG, HDL, TRG, SBP, DBP, WC
Keyhani et al., 2020 (Iran)	Healthy	EX: 54.9 ± 1.0; 27.9 ± 1.3 CON: 56.2 ± 0.7; 27.8 ± 1.2	EX: 10 CON: 10	8 weeks	3 days	Interval (vigorous intensity)	HDL, TRG, SBP, DBP
Latosik et al., 2014 (N/A)	Hypertensive	EX: N/A; 28.2 ± 5.8 CON: N/A; 28.2 ± 4.5	EX: 15 CON: 10	8 weeks	N/A	Continuous (light-vigorous)	HDL, TRG, SBP, DBP, WC
Lee et al., 2012 (Korea)	Obese	EX: 54.8 ± 2.8; 25.1 ± 1.6 CON: 54.3 ± 2.9; 25.2 ± 1.7	EX: 8 CON: 8	16 weeks	3 days	Continuous (light intensity)	FBG, HDL, TRG, SBP, DBP, WC
Lee et al., 2021 (Korea)	Obese	EX: 56.0 ± 2.9; 25.8 ± 2.0 CON: 57.5 ± 2.9; 25.5 ± 1.7	EX: 12 CON:12	16 weeks	5 days	Continuous (light-vigorous intensity)	HDL, TRG
Lesser et al., 2016 (Canada)	Healthy	EX: 56.4 ± 6.9; 29.9 ± 3.5 CON: 57.7 ± 6.1; 28.9 ± 3.5	EX: 23 CON: 26	12 weeks	3 days	Continuous (light-vigorous intensity)	FBG, WC
Libardi et al., 2012 (Brazil)	Healthy	EX: 53.7 ± 3.7; 26.1 ± 3.0 CON: 51.2 ± 6.4; 25.9 ± 2.3	EX: 12 CON: 12	16 weeks	3 days	Resistance (moderate-vigorous intensity)	HDL, TRG
Marcus et al., 2009 (USA)	Healthy	EX: 56.3 ± 6.4; 28.5 ± 3.7 CON: 53.2 ± 6.5; 32.2 ± 4.0	EX: 10 CON: 6	12 weeks	3 days	Resistance (light-moderate)	WC
Miyaki et al., 2012 (Japan)	Healthy	EX: 60.0 ± 6.0; 22.2 ± 2.0 CON: 60.0 ± 7.0; 22.4 ± 2.6	EX: 11 CON: 11	8 weeks	3-5 days	Continuous (light-moderate intensity)	HDL, TRG, SBP, DBP
Moreau et al., 2001 (USA)	Borderline to stage 1 hypertensive	EX: 53.0 ± 7.7; N/A CON: 55.0 ± 3.0; N/A	EX: 15 CON: 9	24 weeks	7 days	Continuous (moderate intensity)	FBG, SBP, DBP
Neves et al., 2017 (Brazil)	Healthy	EX: 58.6 ± 3.9; 27.1 ± 3.7 CON: 57.7 ± 4.8; 27.5 ± 4.6	EX: 27 CON: 19	16 weeks	3 days	Combined (moderate intensity)	FBG, TRG
Nunes et al., 2016 (Brazil)	Healthy	EX: 62.0 ± 10.8; 27.4 ± 7.7 CON: 60.0 ± 7.8; 32.4 ± 6.3	EX: 11 CON: 11	16 weeks	3 days	Resistance (moderate intensity)	HDL, TRG, WC
Rezende Barbosa et al., 2019 (Brazil)	Healthy	EX: 60.0 ± 4.5; 27.3 ± 4.2 CON: 58.5 ± 4.8; 27.6 ± 4.8	EX: 19 CON: 20	18 weeks	3 days	Continuous (moderate intensity)	SBP, DBP

Table 4.2 (continued)

Sénéchal et al., 2012 (Canada)	Dynapenic-obese	62.6 ± 4.1*; N/A	EX: 10 CON: 10	12 weeks	3 days	Resistance (vigorous intensity)	FBG, HDL, TRG, SBP, DBP, WC
Seo et al., 2010 (Korea)	Healthy	EX: 54.0 ± 3.6; 24.0 ± 1.9 CON: 58.0 ± 4.2; 24.0 ± 2.6	EX: 8 CON: 7	12 weeks	3 days	Continuous (vigorous intensity)	FBG, HDL, TRG, SBP, DBP, WC
Son & Park, 2021 (Korea)	Obese	EX: 68.2 ± 1.6; 26.7 ± 3.2 68.2 ± 1.4; 27.1 ± 1.4	EX: 18 CON: 17	12 weeks	3 days	Resistance (light-moderate intensity)	FBG, HDL, TRG, SBP, DBP, WC
Son et al., 2017 (Korea)	Stage 1 hypertensive	EX: 76.0 ± 5.0; 22.8 ± 0.7 CON: 74.7 ± 2.0; 24.1 ± 0.2	EX: 10 CON:10	12 weeks	3 days	Combined (light-moderate intensity)	SBP, DBP
Staffileno et al., 2001 (USA)	Hypertensive	EX: 57.1 ± 8.7; 31.1 ± 4.8 CON: 62.3 ± 8.7; 31.9 ± 5.7	EX: 9 CON: 9	8 weeks	5 days	Continuous (moderate intensity)	SBP, DBP
Trabka et al., 2014 (N/A)	Obese	EX: N/A; 31.6 ± 4.1 CON: N/A; 31.7 ± 4.9	EX: 23 CON: 21	10 weeks	3 days	Combined (moderate-vigorous)	HDL, TRG, WC
Van Gemert et al., 2015 (Netherlands)	Healthy	EX: 58.9 ± 4.6; 26.6 ± 2.9 CON: 58.4 ± 4.2; 27.3 ± 3.6	EX: 96 CON: 93	12 months	2 days	Combined (moderate - vigorous)	FBG
Ward et al., 2020 (Sweden)	Healthy	EX: 55.7 ± 5.1; 28.1 ± 3.9 CON: 55.4 ± 5.0; 26.7 ± 3.6	EX: 26 CON: 29	15 weeks	3 days	Resistance (moderate intensity)	HDL, TRG
Wong et al., 2018 (Korea)	Stage II hypertensive	EX: 59.0 ± 1.0; 24.2 ± 0.8 CON: 59.0 ± 1.0; 23.8 ± 0.8	EX: 21 CON: 20	12 weeks	5 days	Combined (light-moderate intensity)	SBP, DBP
Wong et al., 2019 (Korea)	Stage II hypertensive	EX: 74.0 ± 4.0; 26.0 ± 2.8 CON: 73.0 ± 4.0; 26.9 ± 2.9	EX: 52 CON: 48	20 weeks	3-4 days	Continuous (light-moderate intensity)	SBP, DBP
Wooten et al., 2011 (USA)	Obese	EX: 64.4 ± 0.7; 31.0 ± 0.5 CON: 67.0 ± 0.6; 34.0 ± 1.3	EX: 12 CON: 9	12 weeks	3 days	Resistance (moderate intensity)	HDL, TRG

Data expressed as mean ± SD. [a,b] denotes sub-studies; *denotes combined value of participants; N/A: not applicable as not mentioned; BMI: body mass index; EX: exercise group; CON: control group; MetS: Metabolic Syndrome; T2D: type 2 diabetes; FBG: fasting blood glucose; HDL: high-density lipoprotein; TRG: triglycerides; SBP: systolic blood pressure; DBP: diastolic blood pressure.

4.4.4 Meta-analysis

4.4.4.1 Waist Circumference

The pooled meta-analysis of the 21 studies that included WC as an outcome suggest a large effect size of exercise training significantly reducing WC by 2.62 cm (95% CI: -3.39 to -1.86 cm; p < 0.001). I^2 demonstrated large heterogeneity present between studies (I^2 = 74%, p < 0.001) (Figure 4.2). Sub-group analyses for exercise training intensities, modalities and duration were conducted and are presented in Table 4.4.

The different exercise training intensities showed significant reductions in WC for light-moderate intensity (MD: -3.49 cm; 95% CI: -5.15 to -1.82 cm; p < 0.001; n = 3), moderate intensity (MD: -3.66 cm; 95% CI: -5.61to -1.72 cm; p < 0.001; n = 8), light-vigorous intensity (SMD: -4.00 cm; 95% CI: -6.91 to -1.10 cm; p = 0.007; n = 2).

Likewise, the different exercise training modalities showed significant reductions for continuous training (MD: -1.74cm; 95% CI: -2.36 to -1.12 cm; p < 0.001; n = 8), resistance training (MD: -3.37 cm; 95% CI: -5.83 to -0.91 cm; p = 0.007; n = 6) and combined training (MD: -2.84 cm; 95% CI: -3.88 to -1.80 cm; p < 0.001; n = 7).

Exercise training duration showed significant reductions with short term (MD: -2.18 cm; 95% CI: -4.15 to -0.21 cm; p = 0.03; n = 3), long term (MD: -2.77 cm; 95% CI: -3.83 to -1.71 cm; p < 0.001; n = 12) and very long-term exercise training (MD: -2.55 cm; 95% CI: -3.99 to -1.12 cm; p < 0.001; n = 6). I^2 was significantly reduced after sub-group analyses (intensity: 42.0%; modality: 53.0%; duration: 0%).

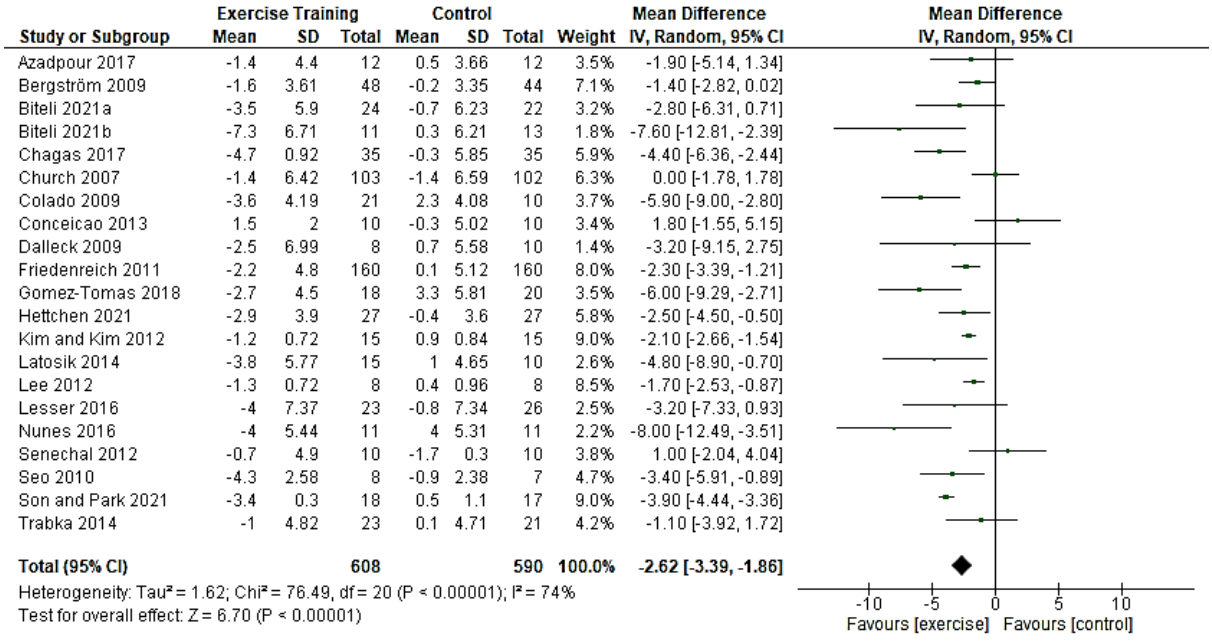

Figure 4.2 Forest plot of randomised controls trials investigating the effect of exercise training vs control on waist circumference using the random effects model. There are a total of 21 studies reporting changes in waist circumference (cm). Negative values favour exercise intervention on the left side. 95% CI: 95% confidence interval; MD: mean difference; SD: standard deviation.

4.4.4.2 Triglycerides

Of the 25 studies including measurements of TRG, the pooled meta-analysis showed exercise training had a small effect reducing TRG by 0.40 mmol/L (95% CI: -0.71 to -0.09 mmol/L; p = 0.01). I^2 demonstrated large heterogeneity present between studies (I^2 = 81%, p < 0.001) (Figure 4.3).

The different exercise training intensities showed reductions in TRG for moderate intensity (SMD: -0.54 mmol/L; 95% CI: -1.05 to -0.02 mmol/L; p = 0.04; n = 10). In addition, different exercise training modalities showed reductions for combined training (SMD: -1.08 mmol/L; 95% CI: -1.86 to -0.30 mmol/L; p = 0.007; n = 6) and exercise training duration showed reductions with short term (SMD: -0.96 mmol/L; 95% CI: -1.66 to -0.26 mmol/L; p = 0.007; n = 5). Sub-group analyses revealed no heterogeneity for intensity (I^2 = 0%), a slight increase for modality (I^2 = 81.8%) and slight decrease for duration (I^2 = 71.1%).

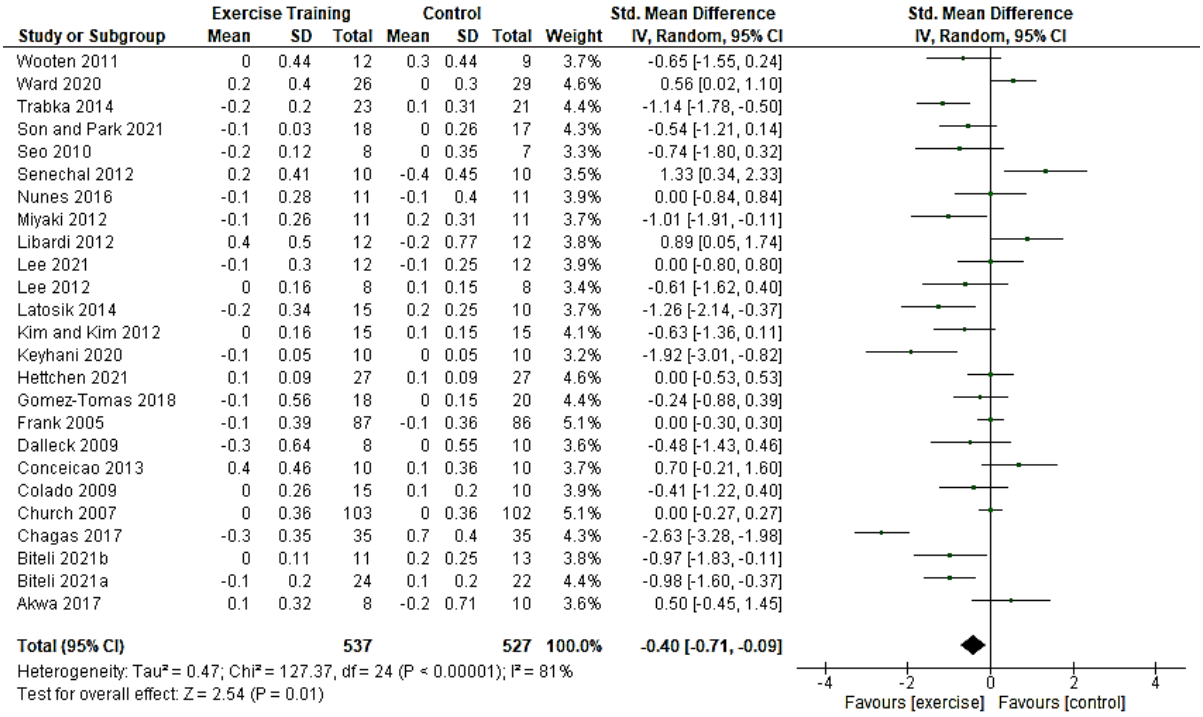

Figure 4.3 Forest plot of randomised controls trials investigating the effect of exercise training vs control on triglycerides using the random effects model. There are a total of 25 studies reporting changes in triglycerides (mmol/L). Negative values favour exercise intervention on the left side. 95% CI: 95% confidence interval; SMD: standardised mean difference; SD: standard deviation.

4.4.4.3 High-density Lipoprotein

Of the 26 studies that included HDL, the pooled meta-analysis showed exercise training had a large effect increasing HDL by 0.84 mmol/L (95% CI: 0.41 to 1.27 mmol/L; p < 0.001). I^2 demonstrated large heterogeneity present between studies (I^2 = 90%, p < 0.001) (Figure 4.4). The different exercise training intensities showed increases in HDL for light-moderate intensity (SMD: 1.97 mmol/L; 95% CI: 0.46 to 3.48 mmol/L; p = 0.01; n = 5). In addition, different exercise training modalities showed increases in HDL for continuous training (SMD: 1.12 mmol/L; 95% CI: 0.20 to 2.03 mmol/L; p = 0.02; n = 9) and resistance training (SMD: 0.96 mmol/L; 95% CI: 0.07 to 1.84 mmol/L; p = 0.04; n = 9).

Exercise training duration showed improvements with short term (SMD: 1.04 mmol/L; 95% CI: 0.00 to 2.07 mmol/L; p = 0.05; n = 5) and long term (SMD: 0.81 mmol/L; 95% CI: 0.29 to 1.33 mmol/L; p = 0.002; n = 16). Sub-group analyses revealed no heterogeneity for intensity and duration (I^2 = 0%), and a slight decrease for modality (I^2 = 87.7%).

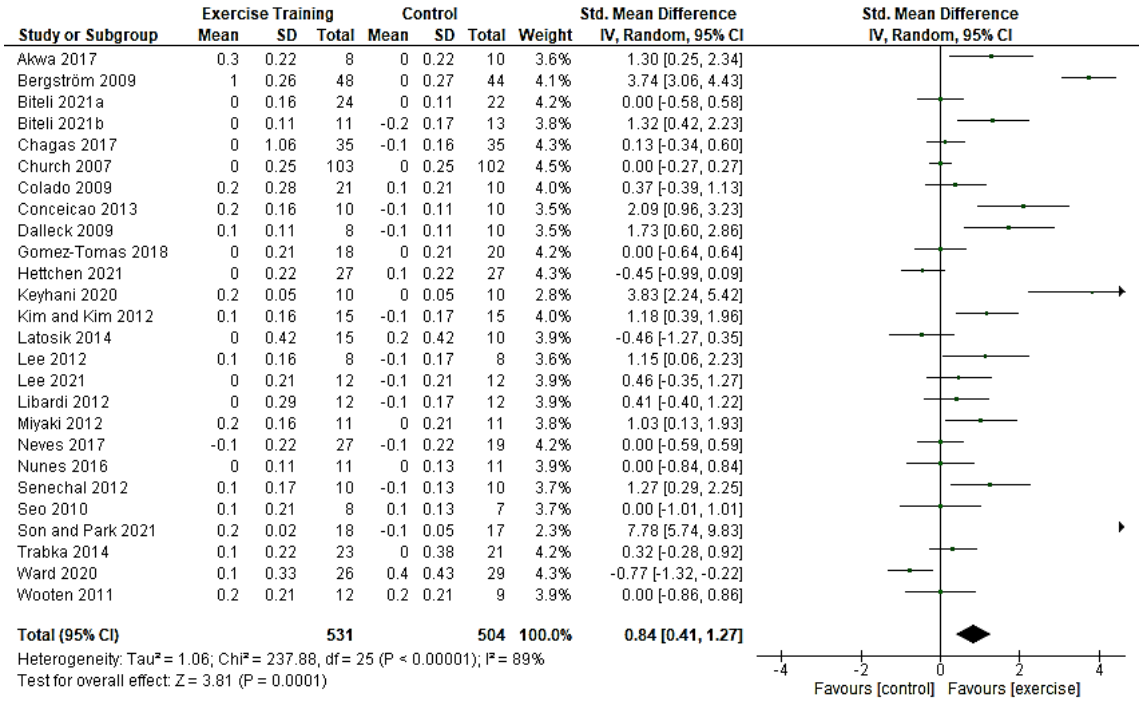

Figure 4.4 Forest plot of randomised controls trials investigating the effect of exercise training vs control on HDL using the random effects model. There are a total of 26 studies reporting changes in HDL (mmol/L). Positive values favour exercise intervention on the right side. Data are reported as SMD (95% CI). HDL: high-density lipoprotein; 95% CI: 95% confidence interval; SMD: standardised mean difference; SD: standard deviation.

4.4.4.4 Fasting Blood Glucose

Of the 20 studies including FBG, the pooled meta-analysis showed exercise training had a small effect decreasing FBG by -0.38 mmol/L (95% CI: -0.60 to -0.16 mmol/L; p < 0.001). I^2 demonstrated large heterogeneity present between studies (I^2 = 63%, p < 0.001) (Figure 4.5).

The different exercise training intensities showed a reduction in glucose with moderate intensity (SMD: -0.54 mmol/L; 95% CI: -0.85 to -0.24 mmol/L; p < 0.001; n = 9), In addition, different exercise training modalities showed a significant reduction in FBG with combined training (SMD: -0.59 mmol/L; 95% CI: -1.01 to -0.16 mmol/L; p = 0.007; n = 7) and exercise training duration showed reductions with long term (SMD: -0.60 mmol/L; 95% CI: -0.90 to -0.31 mmol/L; p < 0.001; n = 13). Sub-group analyses revealed no heterogeneity for intensity (I^2 = 0%), slight decrease for modality (I^2 = 62.7%) and an increase for duration (I^2 = 83.4%).

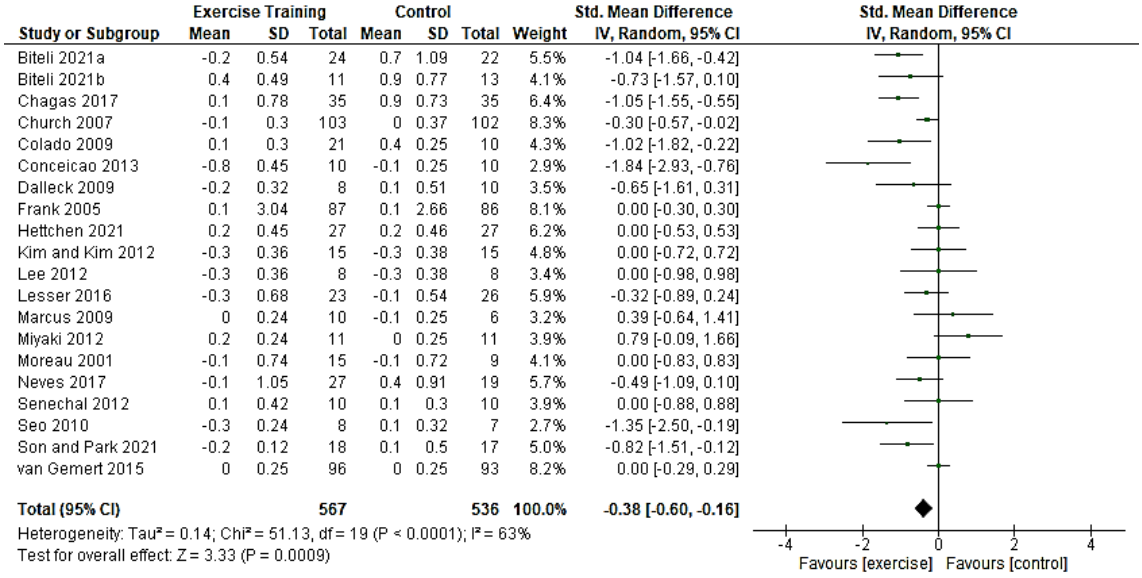

Figure 4.5 Forest plot of randomised controls trials investigating the effect of exercise training vs control on fasting blood glucose using the random effects model. There are a total of 20 studies reporting changes in glucose (mmol/L). Negative values favour exercise intervention on the left side. 95% CI: 95% confidence interval; SMD: standardised mean difference; SD: standard deviation.

4.4.4.5 Systolic Blood Pressure

Of the 23 studies including SBP, the pooled meta-analysis showed exercise training had a large effect decreasing SBP by 5.95 mmHg (95% CI: -7.98 to -3.92 mmHg; p < 0.001). I^2 demonstrated large heterogeneity present between studies (I^2 = 99%, p < 0.001) (Figure 4.6).

The different exercise training intensities showed significant reductions in SBP with light-moderate intensity (MD: -822 mmHg; 95% CI: -11.79 to -4.65 mmHg; p < 0.001; n = 7) and moderate intensity (MD: -5.44; 95% CI: -8.38 to -2.50 mmHg; p < 0.001; n = 9). In addition, different exercise training modalities showed reductions in SBP with continuous training (MD: -7.53 mmHg; 95% CI: -9.95 to -5.10 mmHg; p < 0.001; n = 13) and combined training (MD: -7.28 mmHg: 95% CI: -10.14 to -4.41 mmHg; p < 0.001; n = 4).

Exercise training duration showed reductions with short term (MD: -6.10 mmHg; 95% CI: -7.96 to -4.24 mmHg; p < 0.001; n = 6) and long term (MD: -6.90 mmHg; 95% CI: -9.60 to -4.21 mmHg; p < 0.001; n = 13). I^2 was significantly decreased after sub-group analyses (intensity: 0%; modality: 54.1%; duration: 0%).

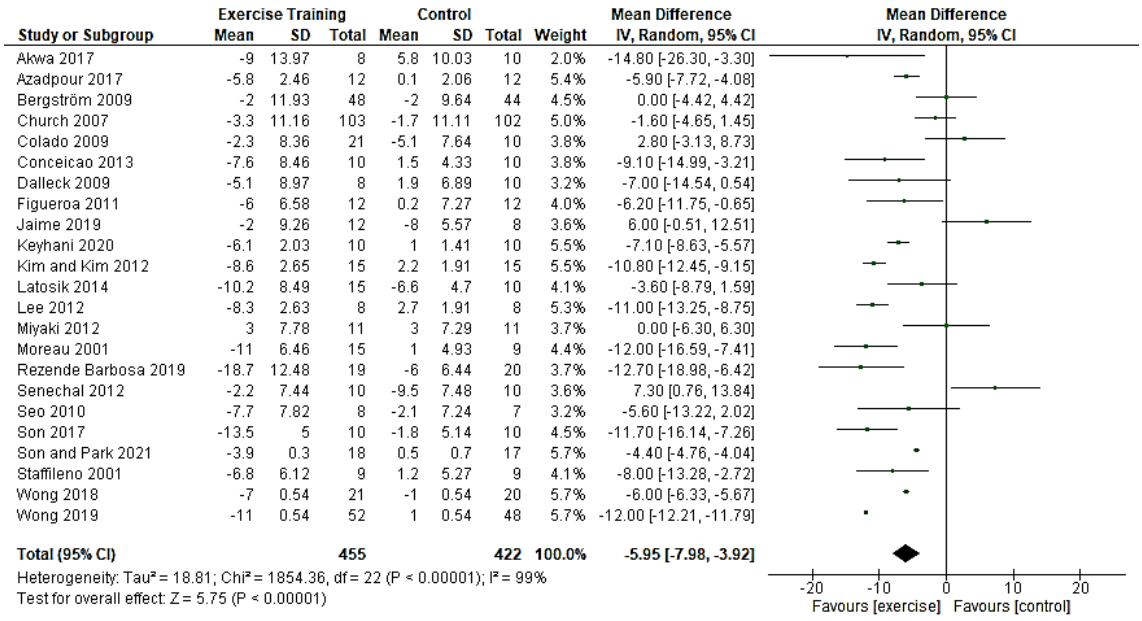

Figure 4.6 Forest plot of randomised controls trials investigating the effect of exercise training vs control on SBP using the random effects model. There are a total of 23 studies reporting changes in SBP (mmHg). Negative values favour exercise intervention on the left side. 95% CI: 95% confidence interval; MD: mean difference; SBP: systolic blood pressure; SD: standard deviation.

4.4.4.6 Diastolic Blood Pressure

Of the 23 studies including DBP, the pooled meta-analysis showed exercise training had a large effect decreasing DBP by 4.14 mmHg (95% CI: -6.19 to -2.08 mmHg; p < 0.001). I^2 demonstrated large heterogeneity present between studies (I^2 = 100%, p < 0.001) (Figure 4.7).

The different exercise training intensities showed reductions in DBP with light-moderate intensity (MD: -5.98; 95% CI: -9.86 to -2.11 mmHg; p = 0.002; n = 7) and moderate intensity (MD: -3.70; 95% CI: -5.42 to -1.98 mmHg; p < 0.001; n = 9). In addition, different exercise training modalities showed reductions in DBP with continuous training (MD: -4.78 mmHg; 95% CI: -7.41 to -2.16 mmHg; p < 0.001; n = 13) and combined training (MD: -4.16 mmHg: 95% CI: -7.03 to -1.29 mmHg; p = 0.005; n = 4).

Exercise training duration showed reductions with short term (MD: -4.61 mmHg; 95% CI: -7.82 to -1.39 mmHg; p = 0.005; n = 6) and long term (MD: -4.41 mmHg; 95% CI: -7.35 to -1.46 mmHg; p = 0.003; n = 13). I^2 decreased significantly after sub-group analyses for intensity (11.0%) and duration (0%), and slightly decreased for modality (90.6%).

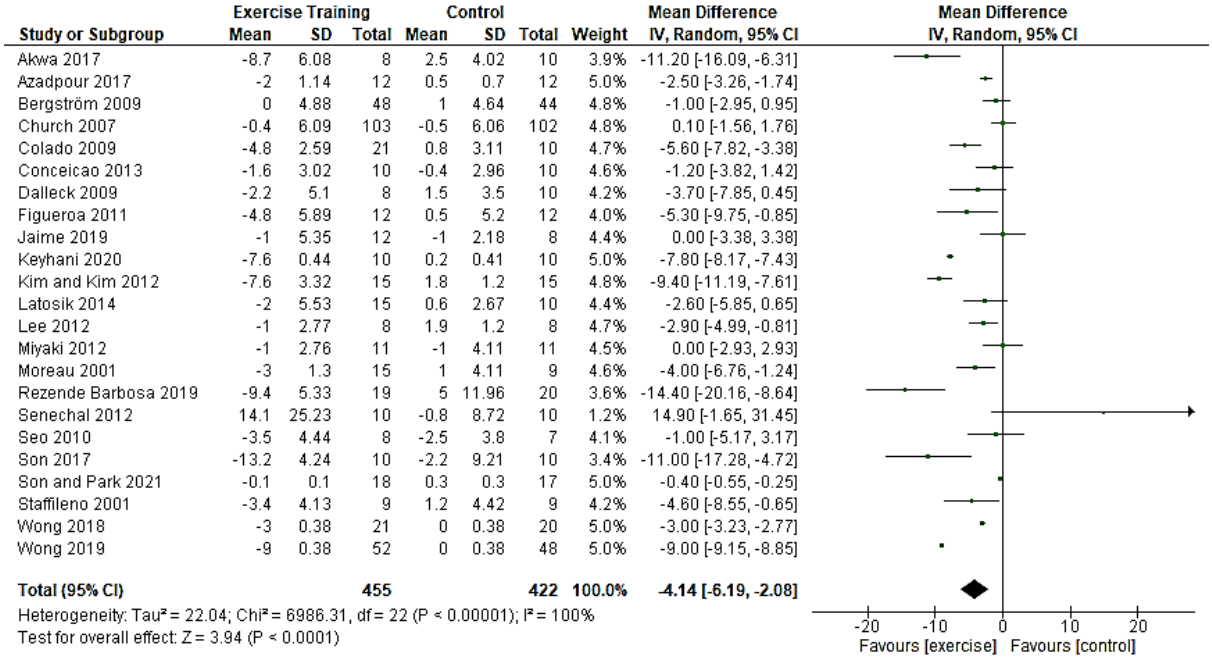

Figure 4.7 Forest plot of randomised controls trials investigating the effect of exercise training vs control on DBP using the random effects model. There are a total of 23 studies reporting changes in DBP (mmHg). Negative values favour exercise intervention on the left side. 95% CI: 95% confidence interval; MD: mean difference; DBP: diastolic blood pressure; SD: standard deviation.

4.4.5 Meta-regression

Across the six meta-analyses, random-effects meta-regression revealed no significant moderator effects of BMI or health status (Table 4.3)

Table 4.3 Meta-regression outcomes.

	Health Status	BMI
WC	β = -0.782; p = 0.49; 95% CI: -3.01 – 1.45	β = -0.100; p = 0.54; 95% CI: -0.42 – 0.22
TRG	β = 0.164; p = 0.35; 95% CI: -0.77 – 1.10	β = -0.053; p = 0.40; 95% CI: -0.18 – 0.07
HDL	β = -0.984; p = 0.22; 95% CI: -2.56 – 0.59	β = -0.128; p = 0.29; 95% CI: -0.36 – 0.11
FBG	β = 0.125; p = 0.69; 95% CI: -0.49 – 0.74	β = -0.039; p = 0.43; 95% CI: -0.14 – 0.06
SBP	β = -2.58; p = 0.27; 95% CI: -7.12 – 1.96	β = -0.08; p = 0.84; 95% CI: -0.84 – 0.68
DBP	β = -1.90; p = 0.26; 95% CI: -5.24 – 1.44	β = -0.133; p = 0.66; 95% CI: -0.73 – 0.46

WC: waist circumference; TRG: triglycerides; HDL: high-density lipoprotein; FBG: fasting blood glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure.

4.5.6 Publication bias and sensitivity analysis

To ascertain publication bias, we used funnel plots and Egger's test. Visual inspection of the funnel plots reveal asymmetry, denoting a certain degree of publication bias (Appendix 2). Egger's test found no evidence of publication bias in WC ($p = 0.16$), TRG ($p = 0.69$), FBG ($p = 0.32$), SBP ($p = 0.32$) and DBP ($p = 0.83$), except for HDL ($p < 0.001$) (Appendix 2). Trim-fill analysis was performed, although no significant changes were found to the data. Sensitivity analysis for pooled analyses revealed that no single trial affected the significance of the SMD, MD or heterogeneity.

Table 4.4 Sub-group analysis of 40 studies.

Groups	WC (cm)				TRG (mmol/L)				HDL (mmol/L)			
	n	MD (95% CI)	*P*	I^2 (%)	n	SMD (95% CI)	*P*	I^2 (%)	n	SMD (95% CI)	*P*	I^2 (%)
Intensity												
Light	1	-1.70 [-2.53, -0.87]	**<0.001***	N/A	1	-0.61 [-1.62, 0.40]	0.24	N/A	1	1.15 [0.06, 2.23]	**0.04***	N/A
Light-to-moderate	3	-3.49 [-5.15, -1.82]	**<0.001***	92%	5	-0.41 [-0.82, 0.01]	0.06	33%	5	1.97 [0.46, 3.48]	**0.01***	92%
Moderate	8	-3.66 [-5.61, -1.72]	**<0.001***	75%	10	-0.54 [-1.05, -0.02]	**0.04***	88%	11	0.56 [-0.09, 1.21]	0.09	92%
Light-to-vigorous	2	-4.00 [-6.91, -1.10]	**0.007***	0%	3	-0.11 [-1.30, 1.07]	0.85	83%	2	-0.00 [-0.90, 0.90]	1	59%
Moderate-to-vigorous	4	-1.29 [-2.93, 0.36]	0.12	45%	2	-0.25 [-2.05, 1.55]	0.78	90%	3	0.83 [-0.10, 1.77]	0.08	74%
Vigorous	3	-1.82 [-4.13, 0.49]	0.12	61%	4	-0.30 [-1.45, 0.85]	0.61	85%	4	1.03 [-0.47, 2.53]	0.18	90%
Modality												
Continuous	8	-1.74 [-2.36, -1.12]	**<0.001***	10%	9	-0.29 [-0.59, 0.02]	0.06	51%	9	1.12 [0.20, 2.03]	**0.02***	90%
Resistance	6	-3.37 [-5.83, -0.91]	**0.007***	82%	9	0.16 [-0.28, 0.59]	0.48	65%	9	0.96 [0.07, 1.84]	**0.04***	90%
Combined	7	-2.84 [-3.88, -1.80]	**<0.001***	42%	6	-1.08 [-1.86, -0.30]	**0.007***	87%	7	0.12 [-0.21, 0.46]	0.47	49%
Interval	0	Not Estimable	N/A	N/A	1	-1.92 [-3.01, -0.82]	**<0.001***	N/A	1	3.83 [2.24, 5.42]	**<0.001***	N/A
Duration												
< 12 weeks	3	-2.18 [-4.15, -0.21]	**0.03***	7%	5	-0.96 [-1.66, -0.26]	**0.007***	69%	5	1.04 [0.00, 2.07]	**0.05***	85%
≥ 12 weeks	12	-2.77 [-3.83, -1.71]	**<0.001***	79%	15	-0.33 [-0.86, 0.19]	0.22	85%	16	0.81 [0.29, 1.33]	**0.002***	85%
≥ 6 months	6	-2.55 [-3.99, -1.12]	**<0.001***	72%	5	-0.04 [-0.21, 0.14]	0.67	0%	5	0.72 [-0.52, 1.96]	0.26	96%

Table 4.4 (continued)

Groups	FBG (mmol/L)				SBP (mmHg)				DBP (mmHg)			
	n	SMD (95% CI)	*P*	I^2 (%)	n	MD (95% CI)	*P*	I^2 (%)	n	MD (95% CI)	*P*	I^2 (%)
Intensity												
Light	1	0.00 [-0.98, 0.98]	1	N/A	2	-2.79 [-19.44, 13.86]	0.74	96%	2	-1.77 [-4.54, 1.01]	0.35	51%
Light-to-moderate	4	0.04 [-0.66, 0.74]	0.91	66%	7	-8.22 [-11.79, -4.65]	**<0.001***	100%	7	-5.98 [-9.86, -2.11]	**0.002***	100%
Moderate	9	-0.54 [-0.85, -0.24]	**<0.001***	64%	9	-5.44 [-8.38, -2.50]	**<0.001***	76%	9	-3.70 [-5.42, -1.98]	**<0.001***	80%
Light-to-vigorous	1	-0.32 [-0.89, 0.24]	0.26	N/A	1	-3.60 [-8.79, 1.59]	0.17	N/A	1	-2.60 [-5.85, 0.65]	0.12	N/A
Moderate-to-vigorous	3	-0.51 [-1.48, 0.46]	0.3	81%	1	-9.10 [-14.99, -3.21]	**0.002***	N/A	1	-1.20 [-3.82, 1.42]	0.37	N/A
Vigorous	2	-0.57 [-1.87, 0.73]	0.39	77%	3	-2.06 [-10.98, 6.87]	0.65	89%	3	-1.81 [-9.40, 5.78]	0.64	88%
Modality												
Continuous	8	-0.12 [-0.32, 0.08]	0.24	15%	13	-7.53 [-9.95, -5.10]	**<0.001***	92%	13	-4.78 [-7.41, -2.16]	**<0.001***	98%
Resistance	5	-0.65 [-1.33, 0.02]	0.06	66%	5	0.15 [-5.42, 5.72]	0.96	87%	5	-1.42 [-4.08, 1.23]	0.29	84%
Combined	7	-0.59 [-1.01, -0.16]	**0.007***	74%	4	-7.28 [-10.14, -4.41]	**<0.001***	52%	4	-4.16 [-7.03, -1.29]	**0.005***	63%
Interval	0	Not estimable	N/A	N/A	1	-7.10 [-8.63, -5.57]	**<0.001***	N/A	1	-7.80 [-8.17, -7.43]	**<0.001***	N/A
Duration												
< 12 weeks	1	0.79 [-0.09, 1.66]	0.08	N/A	6	-7.10 [-8.63, -5.57]	**<0.001***	42%	6	-4.61 [-7.82, -1.39]	**0.005***	97%
≥ 12 weeks	13	-0.60 [-0.90, -0.31]	**<0.001***	47%	13	-6.90 [-9.60, -4.21]	**<0.001***	99%	13	-4.41 [-7.35, -1.46]	**0.003***	100%
≥ 6 months	6	-0.14 [-0.35, 0.07]	0.19	38%	4	-2.80 [-8.55, 2.95]	0.34	86%	4	-2.52 [-5.18, 0.13]	0.06	84%

SMD: standardised mean difference; MD: mean difference; 95% CI: 95% confidence interval; N/A: not applicable; WC: waist circumference; TRG: triglycerides; HDL: high-density lipoprotein; FBG: fasting blood glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure.

4.5 Discussion

This systematic review and meta-analysis evaluated 40 studies, involving 2,132 participants, producing novel exploration to assess the mediating impact of exercise training duration, modality and intensity on MetS risk factors in post-menopausal women. Studies that evaluated exercise training of ≥8 weeks in post-menopausal women who reported at least one MetS risk variable were meta-analysed. Sub-group analyses of exercise intensity, modality and duration were employed to assess the effectiveness of exercise dosing in ameliorating MetS risk. Overall, exercise training was reported to significantly improve MetS risk factors in post-menopausal women, with the largest effect prevalent on SBP and DBP and smallest on FBG. This review also concluded that long term training significantly benefited MetS risk factors except for TRG, and moderate intensity and combined exercise training significantly reduced MetS risk factors, except for HDL.

It is well understood that regular physical activity can be used as a non-pharmacological tool to improve metabolic health. The World Health Organisation (WHO) (World Health Organization (WHO), 2010), ACSM (American College of Sports Medicine, 2014) and the UK Chief Medical Officers (CMO) (Davies et al., 2019) recommend at least 150 minutes of moderate-intensity physical activity or 75 minutes of vigorous-intensity physical activity per week for healthy adults to maintain or improve health (Borg, 1982; Norton et al., 2010). According to the NHS guidelines, this guidance also extends to women undergoing menopause and post-menopause (NHS, 2017). Our findings support current guidance, based on the favourable effects of moderate intensity exercise on MetS risk variables except for HDL, with largest effect on WC, SBP and DBP. However, results for vigorous intensity training were inconclusive due to limited studies. Various studies conducted have evaluated the benefit of exercise training on MetS and cardiovascular risk parameters in middle-aged adults. A meta-analysis by Ashton *et al*. found that medium term (7 – 24 weeks) RT can be effective in improving cardiometabolic health markers in middle-aged adults, specifically in SBP, DBP, HDL, TRG and FBG (Ashton et al., 2020). Ashton *et al*. indicated greater benefit in those with elevated cardiometabolic risk, yet our findings present significant benefit in WC and HDL only in post-menopausal women. However, these inconsistencies could be attributed to population of interest and the limited studies evaluating RT.

Endurance training (any activity that utilises large muscle groups that can be continuously maintained) with supplementation of occasional resistance training is recommended by the ACSM for adults with hypertension (Pescatello et al., 2004). It has been shown that a 10 mmHg reduction in SBP is associated with a 20% risk reduction in major CVD events (Ettehad et al., 2016). This meta-analysis evaluated 7 studies (17.5%) which included post-menopausal women with clinical hypertension, supporting that exercise modalities of continuous and combined exercise training elicited a large effect on SBP and DBP improvements and supports previous published findings (Xi et al., 2021). Similarly, this positive effect was consistent in published literature conducted in both menopausal and post-menopausal women (Loaiza-Betancur et al., 2021). Interestingly, our results showed significant improvements in BP; reductions of 8 mmHg and 6 mmHg for SBP and

DBP respectively, even with light-moderate intensity training. Furthermore, we saw benefits in BP with exercise training in just 8 – 10 weeks. This is supported by a meta-analysis that found hypotensive effects with just a single bout of resistance exercise in healthy adults (Casonatto et al., 2016). This further highlights the benefits of exercise in controlling BP in a relatively short duration in post-menopausal women, and for those who may find a lower intensity of exercise more tolerable.

VAT deposition is known to increase during the menopausal transition due to the decline in oestrogen, which contributes to increased WC and consequently elevates cardiovascular risk (Lovejoy et al., 2008; Samargandy et al., 2021). Collectively, findings indicate that exercise training show effectiveness in reducing WC, with the largest effect particularly with intensities of light-moderate and moderate, modalities of RT, CT, and durations of ≥12 weeks. The effects of exercise training dosage on WC or VAT in post-menopausal women are limited and inconclusive across literature. However, findings are further supported by the only other meta-analysis conducted in post-menopausal women, showing significant reductions in WC with AT of ≥12 weeks (Bueno-Notivol et al., 2017). These findings share similarities with other previous meta-analyses conducted in adults, where they found AE of at least moderate intensity (Chang et al., 2021; Ismail et al., 2012; Vissers et al., 2013) was effective in reducing VAT and WC (Lemes et al., 2018), specifically three times per week for 12 – 16 weeks (Chang et al., 2021). It is understood that WC is surrogate marker for VAT and cannot depict true representation of VAT reductions within this study, which warrants further research required to ascertain the effects of exercise training on VAT in post-menopausal women. Nevertheless, VAT as well as subcutaneous adipose tissue (SAT) are contributors to abdominal obesity which is reflected through WC (Kuk et al., 2005). The ability for exercise to decrease WC are potentially owed to improvements in insulin sensitivity, BG and lipid profiles. Since excess VAT is strongly correlated with impaired glucose and lipid metabolism (Shuster et al., 2014), we theorise to see mediation in these parameters.

TRG and HDL collectively and independently are known to be associated with CVD risk. Hence, the use of TRG to HDL ratio, particularly a ratio >3.5, is used to predict heart disease mortality (Zhan et al., 2014). Additionally, for every 0.026 mmol/L increment in HDL, it has been found to be associated with a 2 – 3% decrease in coronary artery disease risk (Gordon et al., 1989). We found favourable changes in MetS related blood lipids markers that were most apparent with HDL, and the least with TRG. Overall, this is supported and consistent with a review by Wang and Xu, who found HDL sensitivity to aerobic exercise to be higher than that of TRG (Wang & Xu, 2017). A meta-analysis by Wood *et al.* have shown HIIT to be superior MICT in improving HDL levels (Wood et al., 2019). Contrastingly, they found no differences in HIIT nor MICT on the influence of TRG. Although there were limited studies included in our meta-analysis for HIIT, our results were dissimilar for the effects of moderate intensity and continuous training on TRG and HDL levels. We observed a significant decrease in TRG but none in HDL with moderate intensity, of which this was contrasted with continuous training. Moreover, reductions in TRG were seen with CT but not for HDL. It has been proposed through previous studies that exercise duration, intensity and

volume positively correlate with exercise-induced changes in dyslipidaemia, particularly if reductions in TRG are to be achieved (Dunn et al., 1997; Kraus et al., 2002; O'Donovan et al., 2005; Wang & Xu, 2017). Interestingly, sub-group analysis for duration contradicts and showed that improvements in these parameters were diminished for exercise training conducted for ≥12 weeks for TRG, and ≥6 months for HDL. This may be contributed mainly by the high heterogeneity and limited studies, resulting in the inconclusion to ascertain the effect of exercise training on these blood lipids measures.

Exercise training of moderate intensity and CT can have small to moderate mediation in glycaemia, reflected also with exercise training durations of ≥12 weeks. CT comprises of resistance exercises which contribute to muscle strength and hypertrophy (Krzysztofik et al., 2019). Promotion of glucose cell uptake from skeletal muscle during exercise have been proposed to increase insulin sensitivity (Sylow et al., 2016), and this was seen with aerobic exercise of 3 – 4 months in post-menopausal women (Bueno-Notivol et al., 2017). However, CT did not elicit reductions in FBG. Furthermore, caution is required in the interpretation of these findings as participants of included studies for this meta-analysis had no declarations of having impaired glucose or IR. We hypothesise that this modality of exercise training is associated with significant improvements in other MetS parameters and may mediate glycaemic regulation through the prevention of IR development. Further studies are warranted to elucidate exercise training dosage on insulin sensitivity in post-menopausal women.

Overall, this meta-analysis provides further evidence on the superiority of moderate intensity in the modality of CT in providing maximum metabolic benefits. Examples should include exercises involving combined elements of continuous (endurance) training with RT, maintaining levels of moderate intensity as classified with ACSM denoted in Table 4.1

4.5.1 Strengths and limitations

This systematic review and meta-analysis contribute novel findings to literature on the metabolic benefits of exercise training in post-menopausal women. There are many strengths in this study, which are attributed to the inclusion of RCTs only relevant to the meta-analysis and utilising studies with an "intention-to-treat" approach or with ≥80% adherence rate. Sub-group analyses based on exercise training intensities, modalities and duration were also conducted to assess the efficacy of exercise training type on MetS risk variables. However, there are some limitations. Firstly, despite being able to ascertain heterogeneity sources through performing sub-group analyses and meta-regression, there was still a lack of homogeneity across the studies. Participants physical activity status was not included in the meta-regression due to the lack of reporting across numerous studies. Other confounding factors such as diversity in participants' demographics may be a contributing source of heterogeneity. Further work investigating the effects of exercise on MetS risk factors should look to prioritise the influence of participants' characteristics to evaluate the response of exercise on different sub-populations of post-menopausal women. Secondly, due to discrepancies in exercise intervention frequency across

the studies, with several studies not fully reporting the frequency, this was therefore not included in the sub-analyses. Thirdly, we acknowledge the exclusion of a considerably large body of research that have investigated exercise training in post-menopausal women. Post-menopause occurs after menopause and is defined by the cessation of menstruation for at least 1 year. However, to encapsulate the effects of exercise training in post-menopausal women, we only included studies with specific pre-defined post-menopausal status and excluded studies that were ambiguous or did not specify. It was unexpected that this resulted in the loss of a third of eligible studies for inclusion in this meta-analysis (Figure 4.1). It is crucial to specify parameters for certain cohorts of interest in research to draw conclusive findings for these populations. Lastly, certain outcomes of interests were underreported in numerous studies, of which there were no response from contacted authors. To allow future researchers to ascertain the full effects of exercise training in future meta-analyses, we express our concurrence with Hurst *et al.* and Straight *et al.* in the standardisation of reportion exercise training protocols (Hurst et al., 2019; Straight et al., 2016). This encompass mainly training modality, intensity, volume, frequency, duration, adherence rate and fidelity. Consequently, this present review is underpowered and inconclusive for detection of effects for several sub-groups analyses of interest. Therefore, future work that continues to develop precise exercise doses in the prevention or amelioration of MetS risk factors across different populations of post-menopausal women is warranted.

4.6 Conclusion

Physical inactivity and sedentary activity are precursors to metabolic dysfunction that can progress into a plethora of cardiometabolic conditions. The menopausal transition in women results in hormonal imbalances that can further exacerbate these metabolic risks. There is no “one-size fits all” approach. However, this review reinforces the importance of regular physical activity as a non-pharmacological tool in the improvement of MetS risk parameters within post-menopausal women, with significant improvements seen in interventions spanning as short as 8 weeks. Our novel findings further extend the evidence of moderate intensity and combined training in significantly benefitting abdominal obesity, dyslipidaemia, dysglycaemia and hypertension in post-menopausal women. This review also demonstrates that other exercise modalities and intensities performed for at least 8 weeks can elicit benefits in at least one aspect of cardiometabolic risk, seen through improvements in systolic and diastolic blood pressure.

Chapter 5 (GHREX) – Establishing the effects of a home-based, equipment free high-intensity interval training on cardiometabolic risk markers and the feasibility on mediating ghrelin response in post-menopausal women

5.1 Introduction

The findings in Chapter 4 highlight the importance of integrating regular physical activity as a non-pharmacological approach to reduce MetS risk in post-menopausal women, with performing moderate intensity of combined training for a duration of at least twelve weeks to be the most metabolically beneficial. However, barriers to physical activity may limit individuals to fulfil the recommended physical activity guidelines (Koh et al., 2022). It is concerning that approximately 42% of women in the United Kingdom fail to meet the recommended physical activity guidelines (Office for Health Improvement & Disparities (OHID), 2022). Numerous barriers to physical activity are ubiquitous across middle-aged and older adults (Spiteri et al., 2019). Women in these age groups commonly encounter barriers such as lack of enjoyment, time, accessibility, motivation and self-esteem (Tinker et al., 2017). One exercise modality that addresses majority of these barriers is high-intensity interval exercise (HIIE) or high-intensity interval training (HIIT). Despite the expanding evidence highlighting the benefits of HIIT, there remains an apparent dearth of research on this exercise modality. In Chapter 4, out of the 40 studies included in the meta-analysis, only one explicitly investigated HIIT exercise in post-menopausal women (Keyhani et al., 2020).

5.1.1 Mediating Ghrelin with Acute and Chronic Exercise

The investigation into the potential mediation of the ghrelin axis through exercise has gained significant traction, owing to the influence that both have on energy homeostasis and several metabolic pathways. Previous meta-analyses have explored the effects of acute (Anderson et al., 2021; Ouerghi et al., 2021) and chronic exercise-induced ghrelin production (Ouerghi et al., 2021). There is a consensus of an exercise-induced suppression of acyl ghrelin (AG) (Anderson et al., 2021; Ouerghi et al., 2021) (with unaltering total ghrelin (TG) levels) in response to acute bouts of exercise (Ouerghi et al., 2021). Furthermore, Ouerghi *et al.* recently demonstrated that ≥12 weeks of exercise training was associated with either unchanged or decreased AG, with favouring increase in TG that accompanied weight loss in overweight or obese individuals (Ouerghi et al., 2021). Yet, there is a paucity of research on the effects of ghrelin in response to acute and chronic physical exercise training in post-menopausal women (Foster-Schubert et al., 2005; Mason et al., 2015; Steckling et al., 2019; Tremblay et al., 2019). To date, only two studies have investigated the effects of HIIT-induced effects on ghrelin in overweight post-menopausal women (Steckling et al., 2019; Tremblay et al., 2019), with both studies reporting a decrease in fasting plasma AG (Tremblay et al., 2019) and TG (Steckling et al., 2019) following three months of training. Furthermore, although conducted in healthy adults, a meta-analysis by Anderson *et al.* highlighted that acute HIIT exhibits superiority in AG suppression compared to low and moderate intensity exercises, offering insight of the potential in utilising HIIT as a therapeutic approach for dysregulated ghrelin (Anderson et al., 2021). Understanding the mechanisms that mediate exercise-induced changes in ghrelin (both acutely and chronically) could initiate strategies to better improve and maximise the efficacy of exercise in mitigating metabolic dysfunction in post-menopausal women.

5.1.2 Ghrelin, metabolic syndrome and diabetes

In middle-aged (mean age: 51.5 ± 6.0 years) (Ukkola et al., 2006), older (mean age: 72.5 ± 9.0 years) (Langenberg et al., 2005) and elderly (mean age: 77.0 ± 5.9 years) (Serra-Prat et al., 2009) individuals with MetS, fasting TG levels are attenuated compared to individuals of similar age without MetS. Similarly, this effect is also seen in individuals with T2D (Katsuki et al., 2004).

As discussed in section 2.5.4, the intercorrelated relationship between ghrelin, adiposity and MetS highlights the distinct role ghrelin plays in regulating glucose homeostasis and insulin secretion (Chabot et al., 2014). Ghrelin levels are inversely correlated with insulin and glucose levels due to ghrelin's glucoregulatory actions of which is mediated through alterations of downstream effectors (Mani et al., 2019) (Figure 5.1). In healthy individuals in a fasted state, AG reduces insulin sensitivity and inhibits insulin secretion from the pancreatic β-cells, while raising blood glucose levels through increased glucagon secretion from the pancreatic α-cells. The rise in blood glucose subsequently inhibits ghrelin secretion. However, in individuals with comorbidities including obesity and IR where basal AG and TG levels are lower (Wang et al., 2022), it has been theorised that the state of hyperinsulinaemia, in part, play a role in suppressing ghrelin concentrations (Leonetti et al., 2004). A meta-analysis by Zhang *et al.* found negative correlations between ghrelin (AG and TG) and IR in individuals with obesity but normal FBG, which were found to be independent of body mass index (BMI) (Zhang et al., 2018). In line with this, the interplay between the diabetogenic actions of dysregulated ghrelin and whole-body insulin action in diet-induced obesity (DIO) is associated with attenuated insulin sensitivity, thereby contributing to the manifestation of dysglycaemia (Mani et al., 2019). In these individuals, ghrelin secretion in the stomach is impaired, where ghrelin-secreting cells no longer respond effectively to the stimulatory actions of nor-epinephrine or the inhibitory actions of glucose (Uchida et al., 2014). This results in attenuated circulating fasting and post-prandial ghrelin levels. Additionally, DIO reduces ghrelin transport across the blood-brain barrier, impairing the neural circuits in the hypothalamus responsible for feeding mechanism (Banks et al., 2008).

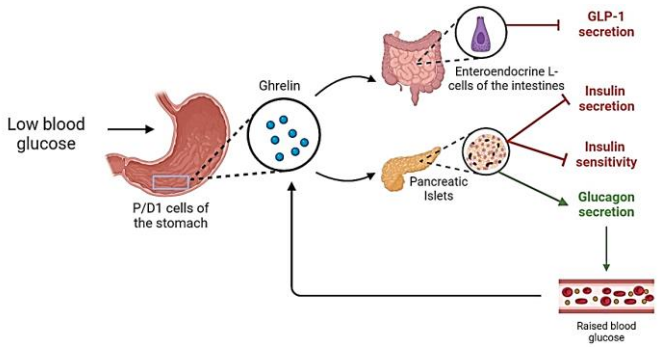

Figure 5.1 **Glucoregulatory actions of ghrelin in fasted, healthy individuals.** In a fasted state where blood glucose levels are low, ghrelin is released from the P/D1 cells of the stomach. Ghrelin reduces insulin sensitivity and inhibits insulin secretion from the pancreas, while raising blood glucose levels through increased glucagon secretion. Simultaneously, GLP-1 secretion is inhibited. The rise in blood glucose inhibits ghrelin secretion. GLP-1: glucagon-like peptide-1; green arrows stimulation; red arrows: inhibition. (***Own image).***

5.2 Aims

The available published literature only encompasses supervised and/or laboratory-based HIIT interventions that are unreflective of real-world implementation in relevant cohorts. In this study, it is hypothesised that chronic equipment-free HIIT (EFHIIT) will improve cardiometabolic risk markers across the total cohort. It is also hypothesised that acute and chronic exposure to EFHIIT will elicit alterations in post-prandial AG and TG. Additionally, these effects will differ between individuals with varying FBG.

This study aims to:

1) Determine the effects of chronic EFHIIT on cardiometabolic risk markers.
2) Explore the feasibility of using EFHIIT to mediate acute and chronic exercise-induced post-prandial ghrelin profiles.
3) Investigate the effects of varying fasting blood glucose on acute and chronic exercise-induced post-prandial ghrelin and cardiometabolic risk markers.

Therefore, it is hypothesised that:

1) Chronic EFHIIT will improve cardiometabolic risk markers across the total cohort.
2) Acute and chronic exposure to EFHIIT will elicit alterations in post-prandial AG and TG.
3) Alterations in post-prandial AG and TG in response to acute and chronic exposure to EFHIIT will differ between individuals with varying FBG.
4) Chronic-EFHIIT response to cardiometabolic risk markers will differ between individuals with varying FBG.

Primary endpoint:

1) Change in cardiometabolic risk markers (FBG, insulin, BP, body composition, lipid profile) following chronic EFHIIT intervention compared to baseline.

Secondary endpoint:

1) Alterations in post-prandial acute and chronic EFHIIT.
2) Correlation between changes in post-prandial ghrelin profile and changes in cardiometabolic risk markers.
3) Differential effects of acute and chronic EFHIIT interventions on post-prandial ghrelin profiles and cardiometabolic risk markers among individuals with varying FBG levels.
4) Evaluation of potential interactions between FBG levels and EFHIIT-induced changes in post-prandial ghrelin and cardiometabolic risk markers.
5) Adherence and compliancy to the interventions.

5.3 Materials and Methods

5.3.1 Ethical Approval

This study was reviewed and was given favourable ethical opinion for conduct by the Solihull Research Ethics Committee (21/WM/0045; IRAS ID: 288904) (Appendix 1). This study was conducted in accordance with the Declaration of Helsinki. All participants have given written and informed consent prior to participation.

5.3.2 Participants

Through contact via email, a total of 43 potential participants expressed interest to the study. They were sent a participant information sheet (PIS) (Appendix 1), denoting the full description of the study. The PIS also contained the inclusion criteria: post-menopausal (defined by cessation of menstruation for at least 12 consecutive month (Davis et al., 2015), aged 45 – 65 years, BMI 25 – 35 kg/m^2, no known diseases, generally well to exercise, physically inactive (defined as not meeting the global health recommendations of physical activity for health of <150 mins/week of moderate-intensity activity, or equivalent) (World Health Organization, 2020). 29 potential participants were excluded; did not meet the inclusion criteria (n = 15), declined/lost contact (n = 9), unable to commit to the study due to lack of time (n = 5). In total, 14 potential participants were then invited on study site for screening. Upon screening, 2 participants did not meet inclusion criteria: low BMI (n = 1), abnormal electrocardiogram ECG and high blood pressure (n = 1). A total of 12 participants were suitable and subsequently recruited to the study after informed consent was given. A flow diagram depicting recruitment of study participants is denoted in Figure 5.2.

4.3.3 Study Design

This was a two-stage, single-armed intervention pilot study with a pre- and post-test design to investigate the mediation of acute and chronic post-prandial ghrelin response with an 8-week home, equipment-free, high-intensity interval training (HEFHIIT) exercise intervention in overweight/obese post-menopausal women. All study visits were held at the Applied Sports Technology, Exercise and Medicine (A-STEM) Research Centre at Swansea University Bay Campus. Eligible participants attended a screening visit, followed by two separate laboratory visits in week 1 and week 8 respectively. The first stage of the study assessed the acute effects of a single bout of EFHIIT on post-prandial AG and TG profile at pre-intervention. The second stage assessed the post-prandial AG and TG profiles in response to a single bout of EFHIIT post-intervention following repeated exposure (24 sessions) to HEFHIIT of 8-weeks. Blood samples via venepuncture were also collected to analyse changes in glycaemia and blood lipid profile before and after intervention. In addition, participants wore a continuous glucose monitoring (CGM) device for an 8-day period each in Week 0 and Week 7. Diet was not monitored to assess the full effect of integrating HEFHIIT only.

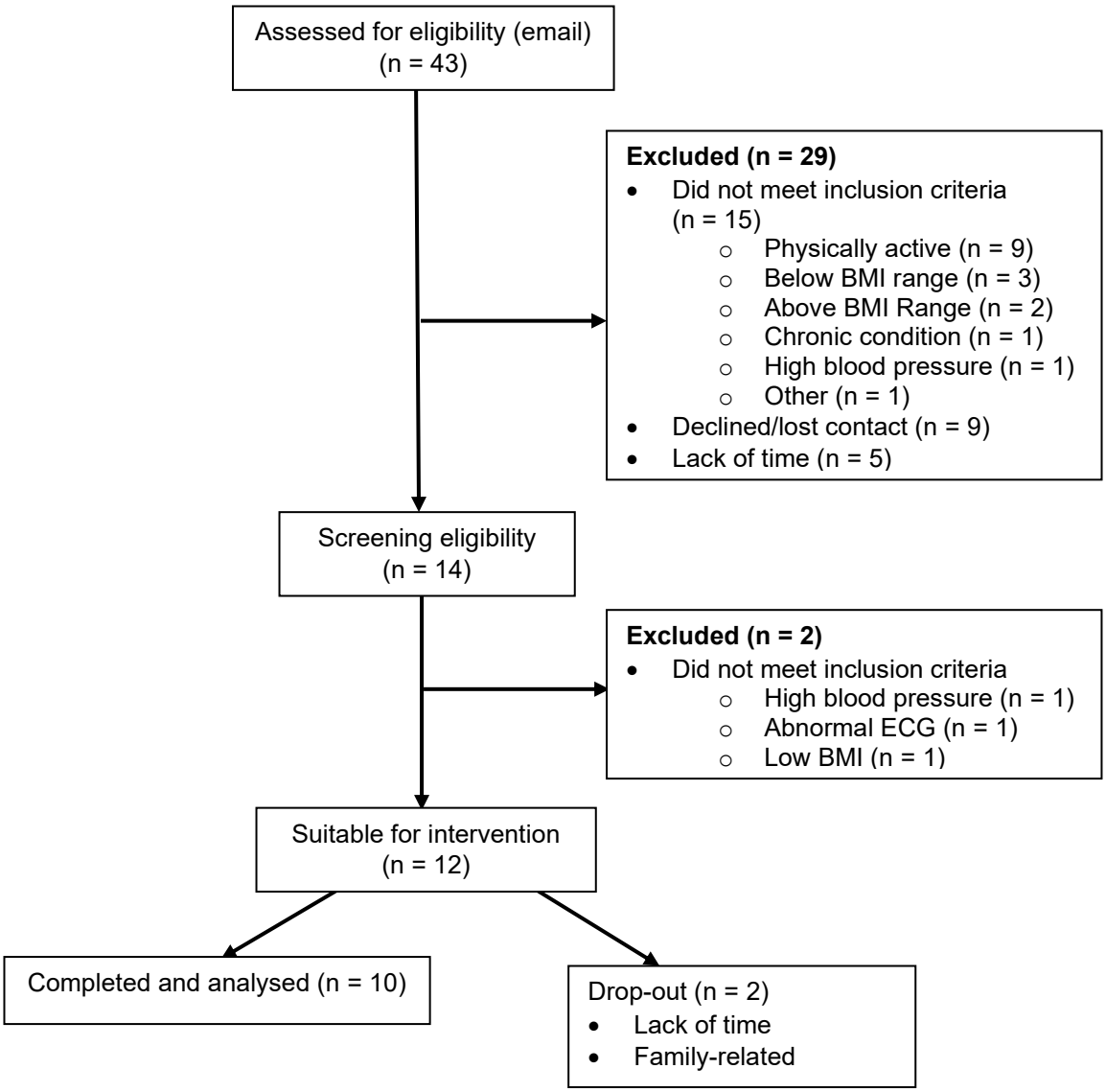

Figure 5.2 Flow diagram of participant recruitment for feasibility study. Some individuals met more than one exclusion criteria. BMI: body mass index; ECG: electrocardiogram.

5.3.4 Experimental Protocol

5.3.4.1 Screening Visit

In week 0, participants attended a screening visit for their eligibility to be assessed. Upon arrival at the laboratory following an overnight fast (≥12 hours) and having abstained from alcohol in the previous 24 hours, participants gave informed consent, completed a menopause questionnaire (Appendix 1) and an International Physical Activity Questionnaire (IPAQ) (Appendix 1). At the end of the screening visit when participants have been deemed eligible, participants completed a Chester Step Test to assess their estimated aerobic capacity. For methodology, please refer to sections 3.3 to 3.4.

5.2.4.2 Laboratory Visits 1 and 2

In weeks 1 and 8, participants arrived following an overnight fast from 2000h (≥12 hours) and having abstained from alcohol in the previous 24 hours. Participants had their anthropometrics and blood pressure taken, followed by a whole-body dual-energy X-ray absorptiometry (DEXA) scan to assess body composition. Additionally, 10mL of fasting blood sample was obtained from the antecubital vein into K_2EDTA BD Vacutainer® tubes.

Participants underwent a comprehensive briefing regarding the education of EFHIIT, involving a range of exercise types and form, target heart rate and rate of perceived exertion (RPE) with the researcher (see section 3.4.5). To isolate the effects of EFHIIT on ghrelin response and eliminate potential confounders such as hunger, participants were fed a standardised meal of butter on a slice of toast (mean ± SD: 111.3 ± 0.7 kcal; 17% protein, 13% fat, 70% carbohydrate). Post-prandial blood capillary samples were collected into a microvette containing EDTA at regular 30-minute intervals at the following time-points: pre-EFHIIT and thirty minutes post-prandial (0-EX); immediately post-EFHIIT (EX); thirty minutes post-EFHIIT (30-EX), to assess plasma AG and TG levels. Microvettes contained a protease inhibitor – AESBF (Millipore-Merck, Burlington, MA, USA) to a final concentration of 1 mg/mL to preserve the octanoyl moiety of ghrelin in the blood (see section 3.6.1). Participants performed a supervised session of an acute bout of EFHIIT at ≥80% of HR_{max} and corresponding to ≥18 Borg RPE scale (Borg, 1982) (Chapter 3, Table 3.2) for twenty minutes at timepoints between 0-EX and EX. A depiction of laboratory visits 1 and 2 is shown in Figure 5.3.

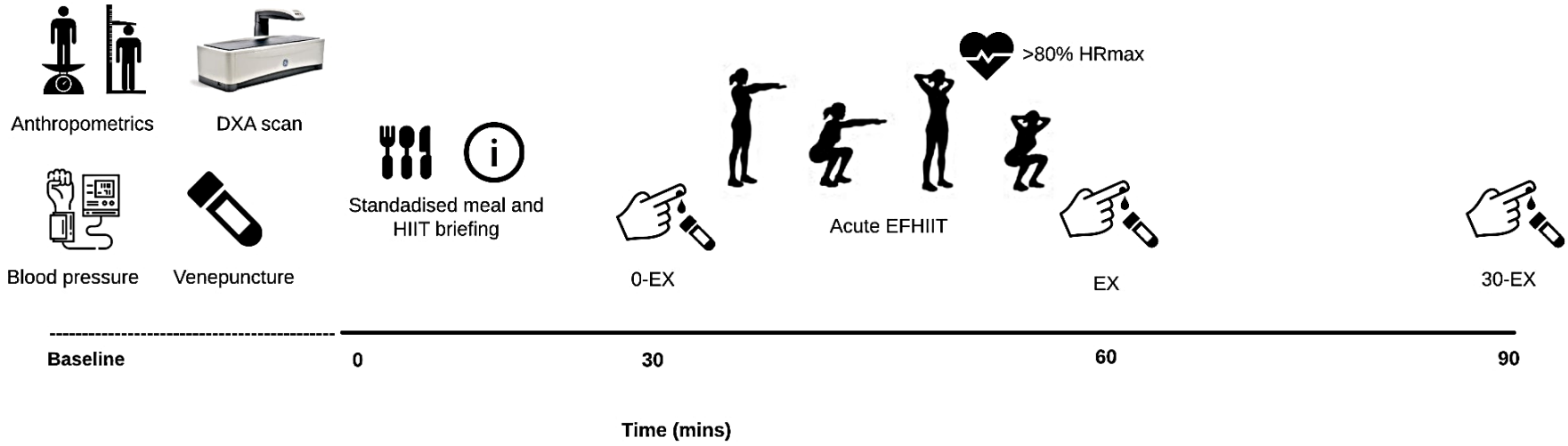

Figure 5.3 Schematic of the study procedures for laboratory visit 1 (week 1) and laboratory visit 2 (week 8). Acute equipment-free high-intensity interval training (EFHIIT) performed at ≥80% maximum heart rate (%HRmax) for twenty minutes following a comprehensive briefing and ten minutes warm up with the researcher. 0-EX: pre-EFHIIT; EX: immediately post-EFHIIT; 30-EX: 30-minutes post-EFHIIT.

5.3.5 Training Protocols

Training was performed three times a week for 8 weeks, with instructions to follow a progressive intensity regime across the 8 weeks. Depending on their preference and confidence in HIIT, participants were asked to follow either: 1) a progressive protocol with the low-intensity intervals progressively decreasing from Week 1 to Week 4 and to complete as many repetitions as possible during the 60s HIIT intervals (Appendix); 2) any HEFHIIT protocol available YouTube videos that suited the participants' preference. These videos were self-chosen on the basis provided that they were suitable and representative of the modality in question and had to meet the following criteria: 1) have "HIIT" in the title of the video; 2) performed without the use of equipment; 3) duration to be no longer than 20 minutes, for emphasis of the time-efficient nature of HIIT.

At the end of each exercise session performed at home, participants were asked to send the details of the session completed to the researcher, including Borg RPE score of the HIIT intervals and the interval durations of the high and low intensities. Each exercise session was also self-recorded by the participants on their Garmin Forerunner 35 device for heart rate data and adherence purposes (Garmin Ltd, United States).

5.3.6 Continuous Glucose Monitoring

A CGM glucose sensor (FreeStyle Libre, Abbott Laboratories Ltd, Chicago, Illinois) were fitted on all participants for eight days during study weeks 0 and 7 to assess the effects of HEFHIIT on glucose variability. At the beginning of the 8-day monitoring periods, a sensor was placed subcutaneously on the posterior upper arm by a trained researcher. On the final day of the eight-day monitoring period at baseline and post-intervention, the CGM device was removed. Interstitial glucose concentrations were measured every minute and stored at 15-minute intervals (FreeStyle Libre, n.d.). Participants were given instructions on the use of the compatible FreeStyle Libre App or FreeStyle Libre glucose reader, which were to scan their sensor with their phone or reader once at least every 8 hours. On the final day of the eight-day monitoring period at baseline and post-intervention, the CGM device was removed. At the end of the study, all retrospective glucose readings were downloaded by research staff via LibreView (LibreView, FreeStyle Libre, Abbott Laboratories Ltd, Berkshire, UK).

Due to a malfunction of the CGM interstitial probe, no baseline data were obtained for one participant and therefore was subsequently excluded from the GCM analysis. For the remaining participants (n = 9), a full 24-hour window (Munan et al., 2020) (i.e., 0300 on day 7 to 0300 on day 8) within the measurement period was analysed for all participants to prevent differences in meal timing affecting the CGM analyses as suggested by Karl *et al.* (Karl et al., 2017). Missing data and gaps in the 24-hour window exceeding 5 minutes were estimated using the following formula (Fonda et al., 2013):

$$k + \frac{glucose\ difference}{number\ of\ 5\ minutes\ increments - 1},$$

where *k* represents the previous glucose value. Daily continuous net glycaemic action (CONGA) of one-hour, two-hour and four-hour (CONGA-1, CONGA-2 and CONGA-4 respectively) were calculated as the standard deviation of the difference between each glucose reading and the previous one-hour, two-hour and 4-hour observations (Mcdonnell et al., 2005). Glycaemic variability measurements were evaluated for time in range (TIR; denoted as 3.9 – 10.0mmol/L) (Battelino et al., 2019), coefficient of variation (CV %), and total area under the curve (t_{AUC}) of 24-h glucose concentrations.

5.3.7 Biochemical Analysis

Both blood capillary samples and fasting venous blood samples were immediately placed on ice following blood draw and processed within 15-minutes of collection. Blood tubes were centrifuged at 4,500 rotations per minute (rpm) for 5 minutes at 4°C to yield plasma. Aliquots of plasma was stored at -80°C until assayed. Fasting HDL, TC, TRG and FBG levels were measured using enzymatic colourimetric method (RANDOX Laboratories Ltd, UK). Glycated haemoglobin (HbA1c) was analysed using a high-performance liquid chromatography (HPLC) method (Tosoh G8 HPLC analyser, Tosoh Bioscience Inc., USA). LDL was estimated using the Friedewald formula (Friedewald et al., 1972). Commercial human-specific ELISA kits were used to quantify insulin concentrations (Invitrogen; Cat# KAQ1251), as well as AG and TG concentrations (Millipore-Merck; EZGRA-88K and EZGRT-89K respectively). The sensitivity of the assays were 0.17 μIU/mL for insulin, 156 pg/ml for TG and 8 pg/ml for AG. All samples were assayed in duplicate. The intraassay coefficient of variation was 7.2% for insulin, 4.5% for AG, and 4.8% for TG.

5.3.8 Sample Size

According to Julious (2005), pilot studies conducted with no prior information have shown that twelve participants are sufficient regarding precision on the mean and variance (Julious, 2005). As the effects of acute and chronic exercise on post-prandial ghrelin profiles have not been formally investigated in post-menopausal women, twelve participants were recruited. The primary outcome of this study was that chronic exercise-induced post-prandial AG and TG would change. A post-hoc power calculation was employed using G*Power (version 3.1) for repeated-measures ANOVA intervention effect for AG and TG, with α level of 0.05 and a power (1 – β) of 0.80. The effect sizes (Cohen's f) of 1.2 and 0.8 respectively.

5.3.9 Normal and Impaired Fasting Blood Glucose Classification

Biochemical analyses showed distinct variations in baseline FBG. Consequently, participants were stratified according to individuals with normal FBG (<6.1 mmol/L; range: 4.2 – 6.0 mmol/L; n = 5), and those with impaired FBG (≥6.1 mmol/L; range: 6.1 – 8.6 mmol/L; n = 5) to establish the effects of glycaemia on the outcomes of the study. This section is elaborated below at section 5.4.2.

5.3.10 Statistical Analysis

All statistical analysis was performed using SPSS 26.0 (SPSS Inc., Chicago, USA). Using the Shapiro-Wilk test, normality of distribution was determined prior to analysis. Statistical significance was set at $p < 0.05$. Normally distributed data are expressed as mean (± standard deviation (SD)) and non-normal distributed data expressed as the median (interquartile range (IQR)). Student paired *t*-test was used to compare within-subjects (pre- and post-intervention) data for normally distributed data, or Wilcoxon signed-rank test for non-normally distributed data. For within-group ghrelin measures, the effect of time on post-prandial ghrelin (timepoints 0-EX, EX and 30-EX) was assessed by one-way repeated measures analysis of variance (ANOVA) and the effect of time and intervention were assessed by 2 x 3 repeated measure ANOVA (pre- vs post- intervention for timepoints 0-EX, EX and 30-EX). Post-hoc multiple comparisons with Bonferroni adjustment were used for any significant interactions. For between-group ghrelin measures, the effect of time, intervention and group was assessed with mixed methods (within-within-between) repeated-measures ANOVA. Post-hoc multiple comparisons with Tukey adjustment were used for any significant interactions. Where appropriate, Greenhouse-Geisser probability levels were employed to adjust for sphericity. Between group, Kruskal-Wallis test was used for non-normally distributed data. To compare between group variables, independent *t*-test was used for normally distributed data, and Mann-Whitney for not normally distributed data. To compare the effects of the intervention between group variables, analysis of covariance (ANCOVA) was employed, adjusted for pre-intervention continuous variables values when these were significantly different between groups. The relationship between intervention changes in AG and TG t_{AUC} with cardiometabolic risk markers was analysed with linear regression analysis. Fasting insulin sensitivity analysis was calculated using the HOMA2 Calculator version 2.2 (University of Oxford, Oxford, UK) (see section 3.8.1). QRISK3 scores were estimated using the QRISK3 algorithm (QRISK3 version 2018.0). Visceral adiposity index (VAI) was calculated with the following equation:

$$\frac{Waist\ circumference\ (cm)}{36.58 + (1.89 \times BMI)} \times \frac{TG\ (mmol/L}{0.81} \times \frac{1.52}{HDL\ (mmol/L)}$$

5.4 Results

The data was split into two distinct categories to explore the investigation of the following factors:

a) Total Study Cohort (n = 10)

b) Effect of Varying FBG (Normal FBG vs Impaired FBG; n = 5 each, respectively)

5.4.1 Total Study Cohort

5.4.1.1 Baseline Characteristics of Participants

Ten participants (age: 58.5 ± 3, BMI: 29.6 ± 2.8 kg/m^2) completed the 8-week intervention. The baseline physical characteristics are shown in Table 5.1. There was a 17% drop-out rate [lack of time (n = 1) and family-related reasons (n = 1)] (Figure 5.2). All women reported generally healthy with no known diseases. Two women were on hormone-replacement therapy (HRT) (8.5 ± 7.8 years) and the average years spent in post-menopause was 8.6 ± 2.9 years.

Table 5.1 Baseline physical characteristics of participants (N = 10).

General Characteristics	***N = 10***
Age (years)	58.5 ± 3.2
Years of menopause (years)	8.6 ± 2.9
Height (m)	1.64 ± 0.05
Weight (kg)	82.9 (68.8 – 86.0)
Waist (cm)	91.6 ± 5.4
Hip (cm)	110.8 ± 5.5
Waist to hip ratio (WHR)	0.8 ± 0.0
BMI (kg/m^2)	29.6 ± 2.8
SBP (mmHg)	130.9 ± 8.9
DBP (mmHg)	80.6 ± 7.6
Estimated Aerobic Capacity (mL/kg/min)	26.3 ± 4.4

Data are presented as mean ± SD and median (IQR) for normally and not normally distributed data respectively. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

5.4.1.2 Body Composition and Cardiometabolic Response to Chronic EFHIIT

Body Composition and Blood Pressure

Analysis on all ten participants revealed that there were no differences in all anthropometrics. Among the eight participants that had pre- and post-intervention body composition assessed via DEXA scans, there were reductions in all body composition markers, yet this data was not statistically significant (Table 5.2).

Following the 8-week intervention, there was a significant reduction of 6 mmHg in SBP (PRE: 130.9 ± 8.9 mmHg vs. POST: 124.9 ± 11.8 mmHg; p = 0.049) and 4 mmHg in DBP (PRE: 80.6 ± 7.6 mmHg vs POST: 76.6 ± 6.9 mmHg, p = 0.004).

Table 5.2 Baseline and changes in body composition and blood pressure in total cohort pre- and post-intervention (N = 10).

	Total Cohort (n = 10)			
	Pre	**Post**	**Δ**	***P***
Weight (kg)	82.9 (68.8 – 86.0)	78.9 ± 8.8	-0.4 ± 1.5	0.51
BMI (kg/m^2)	29.6 ± 2.9	29.3 ± 2.9	-0.3 ± 0.4	0.63
Waist (cm)	91.6 ± 5.4	90.0 ± 6.1	-1.6 ± 3.5	0.19
Hip (cm)	110.8 ± 5.5	109.8 ± 5.4	-1.0 ± 2.2	0.18
Waist to hip ratio (WHR)	0.83 ± 0.02	0.82 ± 0.04	-0.01 ± 0.3	0.53
Body fat[1] %	39.9 ± 4.1	39.2 ± 3.9	-0.7 ± 1.1	0.87
Lean mass[1] (g)	44.9 ± 4.0	44.7 ± 4.1	-0.2 ± 0.8	0.49
VAT mass[1] (g)	663.2 ± 322.9	647.4 ± 323.6	-15.8 ± 14.8	0.31
SAT area[1] (cm^2)	191.5 ± 52.5	187.0 ± 53.1	-4.5 ± 10.8	0.65
Systolic Blood Pressure (mmHg)	130.9 ± 8.9	124.9 ± 11.8	-6.0 ± 8.4	**0.049**
Diastolic Blood Pressure (mmHg)	80.6 ± 7.6	76.6 ± 6.9	-4.0 ± 3.3	**0.004**

Data are presented as mean ± SD for normally distributed data, and median (IQR) for not normally distributed data. BMI: body mass index; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue. [1] represents data analysed in n = 8 for all.

Glycaemic Response

The effect of glycaemic response to HEFHIIT was evaluated in all ten participants. Data analysis revealed that there were no differences found in FBG (6.1 vs. 5.6 mmol/L, p = 0.44), HbA1c (5.6 vs. 5.7 %, p = 0.48), insulin (11.4 vs. 9.7 µU/mL; p = 0.58), HOMA2-%β (88.7 vs. 87.2; p = 0.87), HOMA2-%S (76.3 vs. 74.2; p = 0.71), or HOMA-2IR (1.5 vs. 1.3; p = 0.84) following the 8-week intervention (Table 5.3).

Glucose variability from the CGM was analysed to evaluate the effect of HEFHIIT. Among the nine participants with CGM data, changes in 24-hour blood glucose dynamics shown through t_{AUC} revealed no differences when comparing Week 0 and Week 7 (7600.9 vs. 7613.7 mmol/L x 24-hr; p = 0.95) (Figure 5.4). Similarly, CONGA-1 (0.05 vs 0.06; p = 0.09), CONGA-2 (0.03 vs 0.04; p = 0.09); CONGA-4 (0.03 vs 0.04; p = 0.16), glycaemic variability (18.3 vs 16.5 %CV; p = 0.38) and time in range (95.2 vs 97.0; p = 0.91) did not differ (Table 5.3).

Table 5.3 Baseline and changes in glycaemic measures in participants pre- and post-intervention (N = 10).

	Pre	Post	Δ	*P*
Glucose (mmol/L)	6.1 ± 0.4	5.6 (5.4, 6.6)	0.1 ± 0.7	0.44
HbA1c (%)	5.6 (5.3, 5.9)	5.7 (5.4, 5.9)	0.1 (-0.1, 0.1)	0.48
Insulin (μU/mL)	11.4 ± 1.4	9.7 (7.9, 15.4)	0.6 ± 1.1	0.58
HOMA2-IR	1.5 ± 0.6	1.3 (1.1, 2.1)	0.8 ± 0.5	0.84
HOMA2-%β	88.7 ± 27.6	87.2 ± 12.9	-1.47 ± 28.1	0.87
HOMA2-%S	76.3 ± 32.2	74.2 ± 30.1	-2.1 ± 17.0	0.71
CONGA-1[1]	0.05 ± 0.04	0.06 (0.03, 0.11)	0.03 (-0.01, 0.04)	0.09
CONGA-2[1]	0.03 ± 0.02	0.04 (0.02, 0.07)	0.01 (-0.00, 0.02)	0.09
CONGA-4[1]	0.03 (0.02, 0.03)	0.04 (0.02, 0.07)	0.01 (-0.01, 0.03)	0.16
Glycaemic Variability[1] (%CV)	18.3 ± 4.2	16.5 ± 6.0	-1.7 ± 5.6	0.38
Time in range[1] (%)	95.2 ± 3.7	97.0 (93.5, 98.5)	1.0 (-4.0, 3.0)	0.91
24-hr CGM t_{AUC}[1] (mmol/L x 24-hr)	7600.9 ± 955.3	7613.7 ± 1228.0	12.8 ± 648.3	0.95

Data are presented as mean ± SD and median (IQR). HDL: high-density lipoprotein; LDL: low-density lipoprotein, HbA1c; glycated haemoglobin A1c; HOMA2: homeostatic model assessment of insulin resistance; CONGA: continuous overall net glycaemic action; CV: coefficient variance; CGM: continuous glucose monitor; t_{AUC}: total area under the curve. [1]represents data for n = 9.

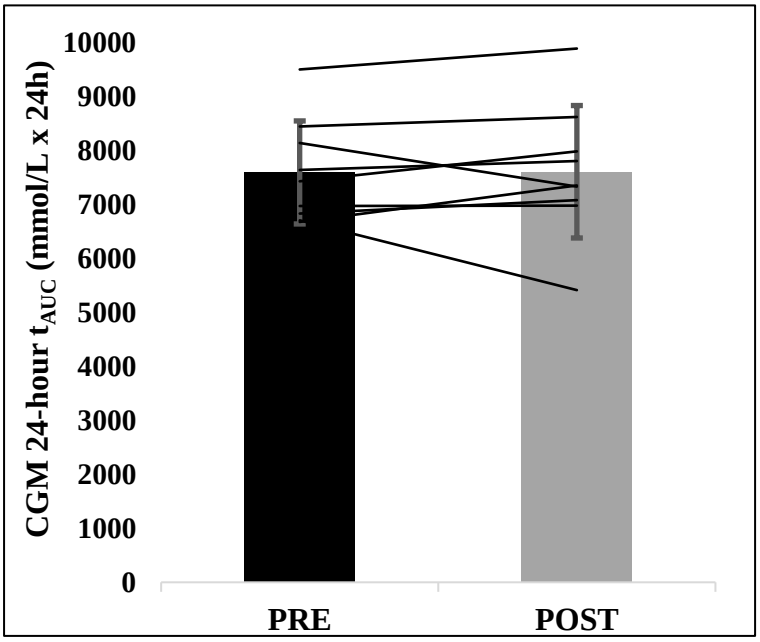

Figure 5.4 Pre- and post-intervention (week 0 vs week 7) continuous glucose monitor t_{AUC} over 24-hour period for all participants (N = 9). Data presented as mean ± standard error of the mean.

Lipid profiles

Analysis of lipid profiles indicated that there were no differences found in all blood lipid markers following the 8-week intervention (TC: 5.4 vs. 5.4 mmol/L; TRG: 1.3 vs.1.2 mmol/L; HDL: 1.7 vs. 1.7 mmol/L; LDL: 3.2 vs. 3.2 mmol/L, $p > 0.05$) (Table 5.4).

Table 5.4 Baseline and changes in lipid markers in participants pre- and post-intervention (N = 10).

	Pre	Post	Δ	*P*
Total Cholesterol (mmol/L)	5.4 ± 0.9	5.4 ± 0.9	0.0 ± 0.1	0.99
Triglycerides (mmol/L)	1.3 ± 0.5	1.2 ± 0.5	-0.1 ± 0.5	0.65
HDL (mmol/L)	1.7 ± 0.4	1.7 ± 0.5	0.0 ± 0.2	0.96
LDL (mmol/L)	3.2 ± 0.9	3.2 ± 0.7	0.0 ± 0.9	0.90

Data are presented as mean ± SD. HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Risk Scores

Baseline and post-intervention risk scores of visceral adiposity index (VAI), QRISK3 scores, MetS-Z severity score and frequency of MetS were analysed to assess the effects of the intervention (Table 5.5). There were no significant changes in these risk scores post-intervention. At baseline, there were 70% (n = 7) of participants that met the MetS diagnosis criteria of which three participants no longer met the MetS criteria at post-intervention.

Table 5.5 Risk scores at baseline and post-intervention in total cohort (N = 10).

	Total Cohort (n = 10)			
	Pre	**Post**	**Δ**	***P***
Visceral Adiposity Index	1.6 ± 0.8	1.6 ± 0.9	-0.1 ± 0.5	0.75
MetS Z-score	2.7 ± 0.7	2.6 ± 0.6	-0.1 ± 0.3	0.35
MetS (n)	7	4	-3	
QRISK3 (%)	5.4 ± 1.5	5.0 ± 1.4	-0.4 ± 0.6	0.10

MetS; metabolic syndrome; QRISK3: cardiovascular risk score 3.

5.4.1.3 Ghrelin Response

First stage (pre-intervention): Acute exercise-induced effect of supervised EFHIIT on post-prandial acyl and total ghrelin

One-way repeated measures analyses were conducted on pre-intervention AG and TG at all three timepoints (baseline (0-EX), EX and 30-EX).

AG: There was a significant main effect for time for AG [$F_{(2,18)} = 10.485$, $p < 0.001$]. Prior to the 8-week HEFHIIT programme, there was a significant exercise-induced suppression in post-prandial AG immediately after performing supervised acute EFHIIT (EX), denoted by a decrease of 43.3% from 0-EX (AG: 0-EX: 336.3 ± 169.0 pg/mL vs EX: 190.8 ± 121.8 pg/mL, $p = 0.004$). This exercise-induced suppression was 38.7% lower during exercise recovery (30-EX) when compared to baseline (30-EX: 206.0 ± 147.7 pg/mL, $p = 0.023$) (Figure 5.5A).

TG: There was a significant main effect for time for TG [$F_{(2,18)} = 8.454$, $p = 0.003$]. Prior to the 8-week HEFHIIT programme, there was a significant exercise-induced suppression in post-prandial TG immediately after performing supervised acute EFHIIT (EX), denoted by a decrease of 25.5% from 0-EX (0-EX: 277.3 ± 170.3 pg/mL vs EX: 206.6 ± 103.7 pg/mL, $p = 0.048$). This reduction was 29.4% lower during exercise recovery (30-EX) when compared to baseline (30-EX: 195.7 ± 98.4 pg/mL, $p = 0.038$) (Figure 5.5C).

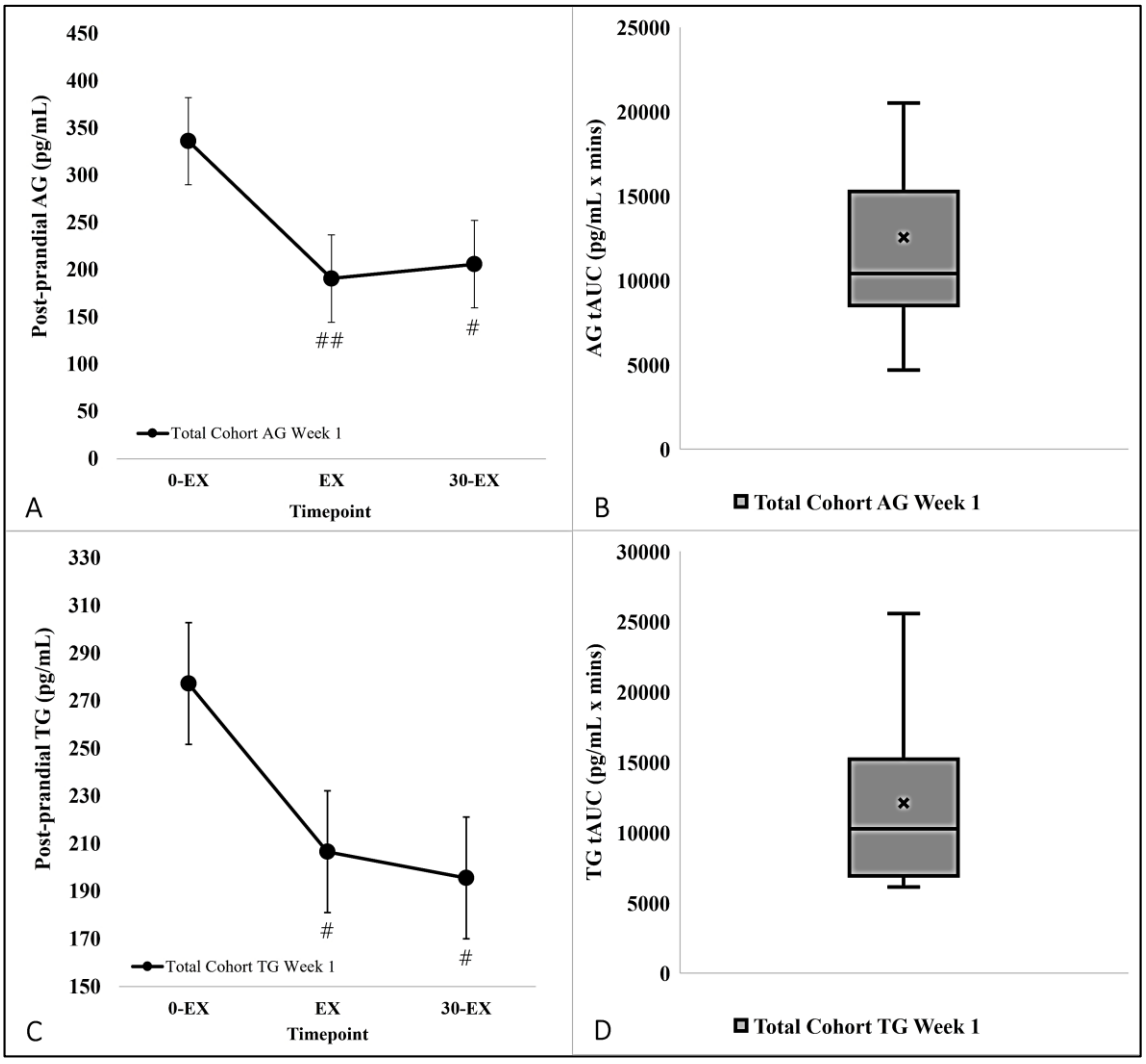

Figure 5.5 A-D: Post-prandial acyl ghrelin (AG) and total ghrelin (TG) response prior and following an acute bout of EFHIIT in Week 1 in N = 10 post-menopausal women. Line graphs show AG (A) and TG (C) at timepoints thirty minutes after feeding during pre-exercise (0-EX), immediately post-exercise (EX), and thirty minutes post-exercise (30-EX) at Week 1. For both AG and TG, repeated measures ANOVA showed significant effects of time (# denotes $p < 0.05$ and ## denotes $p < 0.01$ for timepoints when compared to 0-EX). Box plots show total area under the curve (t_{AUC}) for AG (B) and TG (D) in Week 1. The line within the box plots represents the median, the lower and upper limits of the box are the 25th and 75th percentiles, and the error bars are the 10th and 90th percentiles. Error bars in the line graphs are presented as standard error of the mean.

Stage 2 (post-intervention): Chronic exercise-induced effect of HEFHIIT on post-prandial acyl and total ghrelin

All three AG and TG timepoints were compared pre and post 8-week HEFHIIT intervention to establish the impact of chronic exposure of HEFHIIT on post-prandial ghrelin profiles. Analysis of all time points indicated significant increase in both post-prandial AG t_{AUC} (PRE: 12539.2 ± 6996.3 pg/mL x mins vs POST: 17142.5 ± 9001.4 pg/mL x mins; p = 0.005) and TG t_{AUC} (PRE: 12083.9 ± 6357.8 pg/mL x mins vs POST: 13822.0 ± 3401.5 pg/mL x mins; p = 0.03) following the 8-weeks HEFHIIT programme (Figure 5.6 B&D).

AG: There was a significant main effect for time [$F_{(2,18)}$ = 17.452, p < 0.001), intervention [$F_{(1,9)}$ = 12.741, p = 0.006] but not for time*interaction [$F_{(2,18)}$ = 1.024, p = 0.379]. After the 8-week programme, there was a significant increase by 44.4% in post-prandial AG levels at timepoint baseline (PRE: 336.3 ± 169.0 pg/mL vs POST: 485.6 ± 296.3 pg/mL p = 0.03), and a 50.9% increase in AG levels at timepoint 30-EX, although this was statistically insignificant (PRE: 206.0 ± 147.7 pg/mL vs POST: 310.9 ± 193.7 pg/mL; p = 0.09). There was a significant simple effect for post-intervention AG [$F_{(2,18)}$ = 8.182, p = 0.003]. After the 8-week HEFHIIT programme, there was a significant exercise-induced suppression in post-prandial AG immediately after performing supervised acute EFHIIT (EX), denoted by a 52.0% decrease when compared to baseline (AG: 0-EX: 485.6 ± 296.3 pg/mL vs EX: 233.1 ± 132.5 pg/mL, p = 0.004) (Figure 5.6 A).

TG: There was a significant main effect for time [$F_{(2,18)}$ = 7.267, p < 0.005), intervention [$F_{(1,9)}$ = 6.253, p = 0.034] but not for time*interaction [$F_{(2,18)}$ = 0.997 p = 0.389]. After the 8-week programme, there was a significant increase by 15.3% in TG during at timepoint 30-EX (PRE: 195.7 ± 98.4 pg/mL vs POST: 225.7 ± 103.1 pg/mL; p = 0.02), and a 19.7% increase at timepoint EX (PRE: 206.6 ± 103.7 pg/mL vs POST: 247.4 ± 131.0 pg/mL; p = 0.07), although this was not statistically significant. There was no significant simple effect for post-intervention TG [$F_{(2,18)}$ = 4.048, p = 0.075] (Figure 5.6 C).

Although no significant time*intervention effects were found, a greater exercise-induced suppression observed in AG after the intervention, indicated by an increase in the change in amplitude from baseline to EX (PRE: 43.3% vs POST: 52.0%; p = 0.08). Conversely, the exercise-induced suppression in TG was blunted, as evidenced by a smaller change in amplitude from baseline to EX (PRE: -29.4% vs POST: -14.9%, p = 0.19). However, these were no differences in the findings (Figure 5.6 A&C).

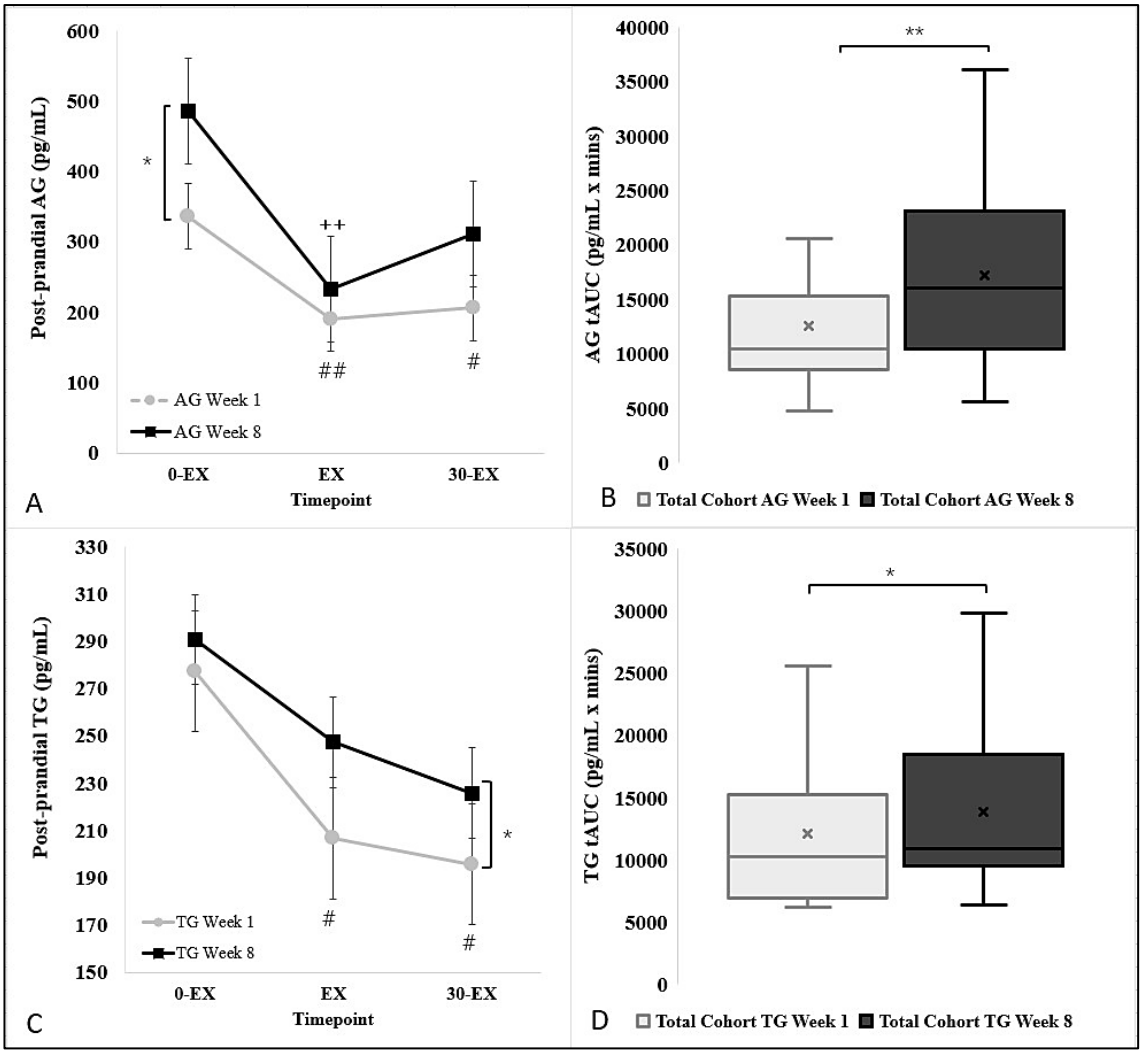

Figure 5.6 A-D: Post-prandial acyl ghrelin (AG) and total ghrelin (TG) response prior and following an acute bout of EFHIIT in Week 1 vs Week 8 in N = 10 post-menopausal women. Line graphs show AG (A) and TG (C) at timepoints thirty minutes after feeding during pre-exercise (0-EX), immediately post-exercise (EX), and thirty minutes post-exercise (30-EX) at Week 1 (—●—) and Week 8 (—■—). For both AG and TG, repeated measures ANOVA showed significant effects on intervention (* denotes $p < 0.05$) and time (# denotes $p < 0.05$ and ++ and ## denotes $p < 0.01$ for timepoints when compared to 0-EX) but no significant effects on intervention*time. Box plots show total area under the curve (t_{AUC}) for AG (B) and TG (D) in Week 1 and Week 8. Paired *t*-tests showed significant effects for both AG and TG (* and ** denotes $p < 0.05$ and $p < 0.01$ respectively). The line within the box plots represents the median, the lower and upper limits of the box are the 25th and 75th percentiles, and the error bars are the 10th and 90th percentiles. Error bars in the line graphs are presented as standard error of the mean.

5.4.1.4 Relationship Between Chronic EFHIIT-induced Changes in Ghrelin and Cardiometabolic Risk Markers

Linear regression was employed to establish the relationship between changes in post-prandial ghrelin profile and changes in cardiometabolic risk markers. No significant associations were found for these variables.

5.4.1.5 Habitual Activity and Compliance to Intervention

The average adherence rate to the intervention was 93% (22 out of 24 sessions). Analysis of the GARMIN logged reports revealed that five participants achieved a 100% adherence rate (24 sessions), while four participants adhered to the exercise program at ≥80 to <100% adherence rate (≥19 to <24 sessions), and the remaining participant had a 62.5% adherence rate (15 sessions). Notably, there was a significant increase in average daily step count in Week 8 compared to Week 0 (Week 0: 5263 ± 1649 steps vs Week 8: 6765 ± 2840 steps; $p = 0.027$).

Participants engaged in various HIIT protocols with instructions to progressively increase intensity across the eight weeks. Self-reported HIIT intervals ranged from 2mins to 20s, and low-intensity intervals ranged from 2min to 10s. Out of the ten participants, three exercised in the HIIT zone (>80% HRmax) for more than 50% of their exercise sessions (64.0 ± 6.7 %HRmax), while the remaining seven participants exercised in moderate intensity zone (60% - 80% HRmax) for more than 50% of their exercise sessions (57.3 ± 9.5 %HRmax). On average, participants reached 99.1 ± 5.7 %HRmax at each session, with three participants reaching >100% of the HRmax (106.5 ± 2.5 %HRmax). The average rating for RPE for the HIIT intervals only were 18.5, with nine out of ten participants exerting ≥17.5 RPE. The remaining participant exerted an average of 16 RPE.

5.4.2 Effect of Varying Fasting Blood Glucose (split cohort)

The interrelationship between ghrelin and glucose homeostasis warrants further investigation on the effect of fasting blood glucose (FBG) on acute and chronic exercised-induced ghrelin and physiological markers with EFHIIT. Participants displayed varying baseline FBG and thus were stratified according to those presenting with baseline normal FBG (<6.1 mmol/L; n = 5) and impaired FBG (≥6.1 mmol/L; n = 5).

5.4.2.1 Baseline Characteristics of Participants

The baseline physical characteristics for those with and without impaired FBG are shown in Table 5.6. There were no differences observed in age, years of menopause, body weight and WC. Baselines DBP among those with impaired FBG was significantly higher than those without (85.4 mmHg vs 75.8 mmHg, p = 0.04).

Table 5.6 Baseline physical characteristics of participants with and without impaired fasting blood glucose.

General Characteristics	***Normal FBG (N = 5)***	***Impaired FBG (N = 5)***	***P***
Age (years)	60 (55.5, 61.0)	58.4 ± 2.9	0.60
Years of menopause (years)	8.4 ± 2.7	8.8 ± 3.3	0.84
Height (m)	1.65 ± 0.06	1.65 ± 0.05	0.56
Weight (kg)	76.5 ± 9.1	85.0 (75.8, 86.1)	0.35
Waist (cm)	90.0 ± 7.0	93.2 ± 3.1	0.38
Hip (cm)	108.3 ± 6.2	113.4 ± 3.8	0.16
Waist to hip ratio (WHR)	0.83 ± 0.03	0.82 ± 0.01	0.59
BMI (kg/m^2)	28.2 ± 2.4	30.9 ± 2.6	0.06
SBP (mmHg)	136. ± 9.1	125.8 ± 5.4	0.06
DBP (mmHg)	85.4 ± 6.5	75.8 ± 5.4	**0.04**
Estimated Aerobic Capacity (mL/kg/min)	25.8 ± 3.7	27.7 ± 5.3	0.53

Data are presented as mean ± SD for normally distributed data, and median (IQR) for not normally distributed data. Between groups, normally distributed data and not normally distributed data were compared with independent *t*-test and Mann-Whitney test respectively. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

5.4.2.2 Body Composition and Cardiometabolic Risk Markers

Body Composition

Comparisons between individuals with normal or impaired FBG revealed similar reductions in weight (-0.2 kg vs -0.3), BMI (-0.1 kg/m^2 vs -0.1 kg/m^2) and WC (-1.7 cm vs -1.4 cm) in both groups, although reductions were non-significant within group. HC reductions were nearly 2-fold in the impaired FBG when compared to the normal FBG group (-1.3 cm vs -0.7 cm), although this was not different between and within group. Post-intervention and delta values for all anthropometrics and body composition did not differ between groups (Table 5.7).

Upon stratification for baseline glycaemia in participants that had pre- and post-intervention body composition assessed via DEXA scans, there were three participants in the normal FBG group and five participants in the impaired FBG group. Body composition variables did not differ between groups. There were no differences in body composition except for VAT mass (-30.7 g, p = 0.046) in the normal FBG group (Table 5.7).

Table 5.7 Baseline and changes in anthropometrics and body composition in participants with and without impaired FBG pre- and post-intervention (n = 10).

	Normal FBG (n = 5)				Impaired FBG (n = 5)				P
	Pre	**Post**	**Δ**	***P***	**Pre**	**Post**	**Δ**	*P*	**(G)**
Weight (kg)	76.5 ± 9.1	76.2 ± 8.4	-0.3 ± 1.4	0.63	85.0 (75.8, 86.1)	81.6 ± 9.3	-0.2 ± 1.6	0.85	0.90
BMI (kg/m^2)	28.2 ± 2.4	28.1 ± 2.3	-0.1 ± 0.5	0.68	30.9 ± 2.6	30.9 ± 2.8	-0.1 ± 0.7	0.81	0.97
Waist (cm)	90.0 ± 7.0	88.3 ± 5.3	-1.7 ± 2.0	0.13	93.2 ± 3.1	91.7 ± 6.9	-1.4 ± 4.8	0.54	0.83
Hip (cm)	108.3 ± 6.2	107.6 ± 5.2	-0.7 ± 2.0	0.49	113.4 ± 3.8	112.0 ± 5.1	-1.3 ± 2.6	0.31	0.95
Waist to hip ratio (WHR)	0.83 ± 0.03	0.82 ± 0.03	-0.01 ± 0.2	0.37	0.82 ± 0.01	0.82 ± 0.04	-0.00 ± 0.04	0.86	0.99
Body fat[1] %	39.9 ± 4.1	39.2 ± 3.9	-0.7 ± 1.1	0.87	39.1 ± 4.8	39.3 ± 4.9	-0.2 ± 1.1	0.72	0.84
Lean mass[1] (g)	44.9 ± 4.0	44.7 ± 4.1	0.2 ± 1.4	0.80	45.4 ± 4.5	45.1 ± 4.2	-0.0 (-0.4, 24.8)	0.22	0.97
VAT mass[1] (g)	581.4 ± 152.4	550.6 ± 154.9	-30.7 ± 11.8	**0.046**	712.2 ± 403.5	705.4 ± 400.0	-6.8 ± 6.8	0.09	**0.015**
SAT area[1] (cm^2)	208.3 ± 59.9	203.2 ± 69.3	-28.4 ± 9.4	0.45	181.5 ± 177.3	159.2 (148.9, 214.8)	-4.2 ± 12.6	0.35	0.95

Data are presented as mean ± SD for normally distributed data, and median (IQR) for not normally distributed data. FBG: fasting blood glucose; BMI: body mass index; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue. [1] represents data analysed in n = 3 for normal FBG and n = 5 for impaired FBG. G: group effect.

Blood Pressure

The effects of glycaemia on BP were analysed in response to the intervention. Individuals without impaired FBG exhibited reductions in both SBP and DBP, although this was not significant (SBP: 136 mmHg vs 134 mmHg, p = 0.61, DBP: 85 mmHg vs 81 mmHg, p = 0.06). Among those with impaired FBG, these individuals exhibited significant reductions of 10 mmHg in SBP (PRE: 126 ± 5 mmHg vs. POST: 115 ± 3 mmHg; p = 0.046) and trending reductions of 4 mmHg in DBP (PRE: 76 ± 6 mmHg vs POST: 72 ± 6 mmHg, p = 0.06). Post-intervention SBP and DBP significantly differed between groups (SBP: p = 0.002; DBP: p = 0.024). As there was a large difference in baseline SBP and DBP, ANCOVA was employed adjusting for baseline values. Further analysis revealed that between groups, change in SBP was significant (-3.2 mmHg vs -10.4 mmHg, p = 0.022), but not in DBP (-4.2 mmHg vs -3.8 mmHg, p = 0.42).

Lipid Profiles

Between and within group analysis of lipid profiles were employed across the two groups (Table 5.8). There were no significant differences observed between and within groups in all lipid markers.

Table 5.8 Baseline and changes in lipid markers in participants with and without impaired FBG pre- and post-intervention.

	Normal FBG (n = 5)				**Impaired FBG (n = 5)**				***P (G)***
	Pre	**Post**	**Δ**	**P**	**Pre**	**Post**	**Δ**	**P**	
TC (mmol/L)	5.6 ± 1.2	5.8 ± 0.8	0.2 ± 1.2	0.75	5.2 ± 0.8	5.1 ± 0.8	-0.2 ± 0.7	0.59	0.26
TRG (mmol/L)	1.8 (0.8, 1.9)	1.3 ± 0.5	-0.2 ± 0.6	0.69	1.2 ± 0.5	1.2 ± 0.5	-0.1 (-0.1, 0.3)	0.56	0.64
HDL (mmol/L)	1.8 ± 0.3	1.9 ± 0.4	0.1 ± 0.1	0.08	1.5 ± 0.4	1.4 ± 0.4	-0.1 ± 0.3	0.42	0.16
LDL (mmol/L)	3.2 ± 1.2	3.3 ± 0.9	0.2 ± 1.3	0.78	3.2 ± 0.7	3.1 ± 0.6	-0.1 ± 0.5	0.70	0.63

Data are presented as mean ± SD for normally distributed data, and median (IQR) for not normally distributed data. Between groups, normally distributed data and not normally distributed data were compared with independent t-test and Mann-Whitney test respectively. There was no significant difference between groups. Within groups, normally distributed data and not normally distributed data were compared with paired *t*-test and Wilcoxon Signed Ranks test respectively. TC: total cholesterol; TRG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein. G: group effect.

Glycaemic Response

Analysis comparing the effects of the intervention in the normal FBG group to the impaired FBG group was conducted. As expected, baseline FBG was significantly higher in the impaired group compared to the normal FBG group (6.9 mmol/L vs 5.2 mmol/L; p = 0.014). However, there were no significant differences in baseline HbA1c (p = 0.09) and insulin levels (p = 0.30) between groups (Table 5.10)

In the impaired FBG group, there was a significant reduction in FBG following the intervention (6.9 mmol/L vs 6.4 mmol/L; p = 0.03). However, there were no differences when comparing pre-intervention to post-intervention values for insulin levels (12.2 μU/mL vs 14.4 μU/mL; p = 0.37), HbA1c (6.0 mmol/L vs 6.0 mmol/L; p = 0.62), HOMA2-IR (1.7 vs 1.9; p = 0.41), HOMA2-%β (67.6 vs 84.2; p = 0.15) or HOMA2-%S (72.3 vs 68.3; p = 0.70).

Similarly, there were no significant changes in all glycaemic markers in the normal FBG group when comparing pre-intervention to post-intervention values (insulin: 10.6 μU/mL vs 9.9 μU/mL, p = 0.29; FBG: 5.2 mmol/L vs 5.6 mmol/L, p = 0.34; HOMA2-IR: 1.4 vs 1.5, p = 0.71; HOMA2-%β: 110.0 vs 92.8, p = 0.19; HOMA2-%S: 80.3 vs 61.2; p = 0.44).

As there was a significant difference in baseline FBG between groups, ANCOVA was employed adjusting for baseline values. Further analysis revealed that between groups, change in FBG was not significant between impaired FBG group and normal FBG group (-0.5 mmol/L vs 0.4 mmol/L, p > 0.05). No other significant differences were found between groups except for delta HOMA2-%β (normal FBG vs impaired FBG: -17.0 vs 16.6, p = 0.046).

The evaluation on the effects of the intervention on glycaemic variability in those with different glycaemic status were analysed. Upon stratification for baseline FBG in participants that had pre- and post-intervention CGM data, there were five participants in the normal FBG group and four participants in the impaired FBG group (Figure 5.7). Results demonstrated that there was a significant decrease in glycaemic variability in women with baseline normal FBG (19.0 %CV vs 17.2 %CV, p = 0.03). Contrastingly, this effect was not present within individuals with impaired FBG (17.3 %CV vs 18.8 %CV, p > 0.05). There were no differences when comparing pre-intervention to post-intervention values for 24-hr CGM t_{AUC}, TIR, CONGA-1, CONGA-2 and CONGA-4 (p > 0.05) between and within groups (Table 5.10).

Table 5.9 Baseline and changes in glycaemic markers in participants with and without impaired FBG pre- and post-intervention.

	Normal FBG (n = 5)				Impaired FBG (n = 5)				P (G)
	Pre	**Post**	**Δ**	**P**	**Pre**	**Post**	**Δ**	**P**	
Glucose[#] (mmol/L)	5.2 ± 0.7	5.6 ± 0.3	0.4 ± 0.8	0.34	6.9 ± 1.0[$]	6.4 ± 1.1	-0.5 ± 0.3	**0.03**	0.59
HbA1c (%)	5.4 ± 0.2	5.4 ± 0.3	0.1 (0.0, 0.1)	0.21	6.0 ± 0.7	6.0 ± 0.6	0.5 ± 1.4	0.62	0.52
Insulin (μU/mL)	10.6 ± 3.8	9.9 ± 2.7	-1.7 (-1.8, 0.7)	0.29	12.2 ± 5.4	14.2 ± 8.1	2.0 ± 4.5	0.37	0.28
HOMA2-IR	1.4 ± 0.5	1.5 ± 0.4	0.1 ± 0.5	0.71	1.7 ± 0.8	1.9 ± 1.1	0.2 ± 0.6	0.41	0.70
HOMA2-%β	110.0 ± 20.5	92.8 ± 9.4	-17.0 ± 23.8	0.19	67.6 ± 13.6[$$]	84.2 ± 15.8	16.6 ± 21.1[$]	0.15	0.48
HOMA2-%S	80.2 ± 29.1	61.2 ± 37.1	4.6 (63.0, 12.9)	0.44	72.3 ± 38.0	68.3 ± 40.4	-4.0 ± 20.3	0.70	0.65
CONGA-1[1]	0.03 (0.01, 0.09)	0.06 ± 0.04	0.01 ± 0.03	0.18	0.05 ± 0.02	0.07 (0.04, 0.79)	0.02 (-0.02, 0.74)	0.27	0.35
CONGA-2[1]	0.03 ± 0.03	0.04 ± 0.02	0.01 ± 0.01	0.15	0.04 ± 0.01	0.04 (0.03, 0.51)	0.01 (-0.01, 0.48)	0.27	0.35
CONGA-4[1]	0.02 (0.02, 0.06)	0.01 ± 0.02	0.00 ± 0.02	0.72	0.03 ± 0.01	0.05 (0.02, 0.31)	0.02 (-0.01, 0.29)	0.14	0.32
Glycaemic Variability[1] (%CV)	19.0 ± 5.3	17.2 ± 4.9	-1.8 ± 1.5	**0.03**	17.3 ± 2.8	18.8 ± 3.5	1.4 ± 4.1	0.27	0.21
Time in range[1] (%)	95.8 ± 2.4	97.6 ± 1.8	1.8 ± 2.8	0.22	94.5 ± 5.3	93.5 (48.8, 97.8)	-2.5 (-42.8, 1.8)	0.47	0.29
24-hr CGM t_{AUC}[1] (mmol/L x 24-hr)	7470.9 ± 640.2	7701.5 ± 769.6	230.6 ± 201.8	0.06	7763.4 ± 1350.5	7503.9 ± 1837.6	-1404.2 ± 2508.9	0.62	0.06

Data are presented as mean ± SD for normally distributed data, and median (IQR) for not normally distributed data. Within groups, normally distributed data were analysed with paired *t*-test and not normally distributed data with Wilcoxon signed ranks test. Between groups, normally distributed data and not normally distributed data were compared with independent *t*-test and Mann-Whitney test respectively. [#] Analysis of covariance employed, adjusting for baseline values. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. [$] denotes significance between groups. G: group effect.

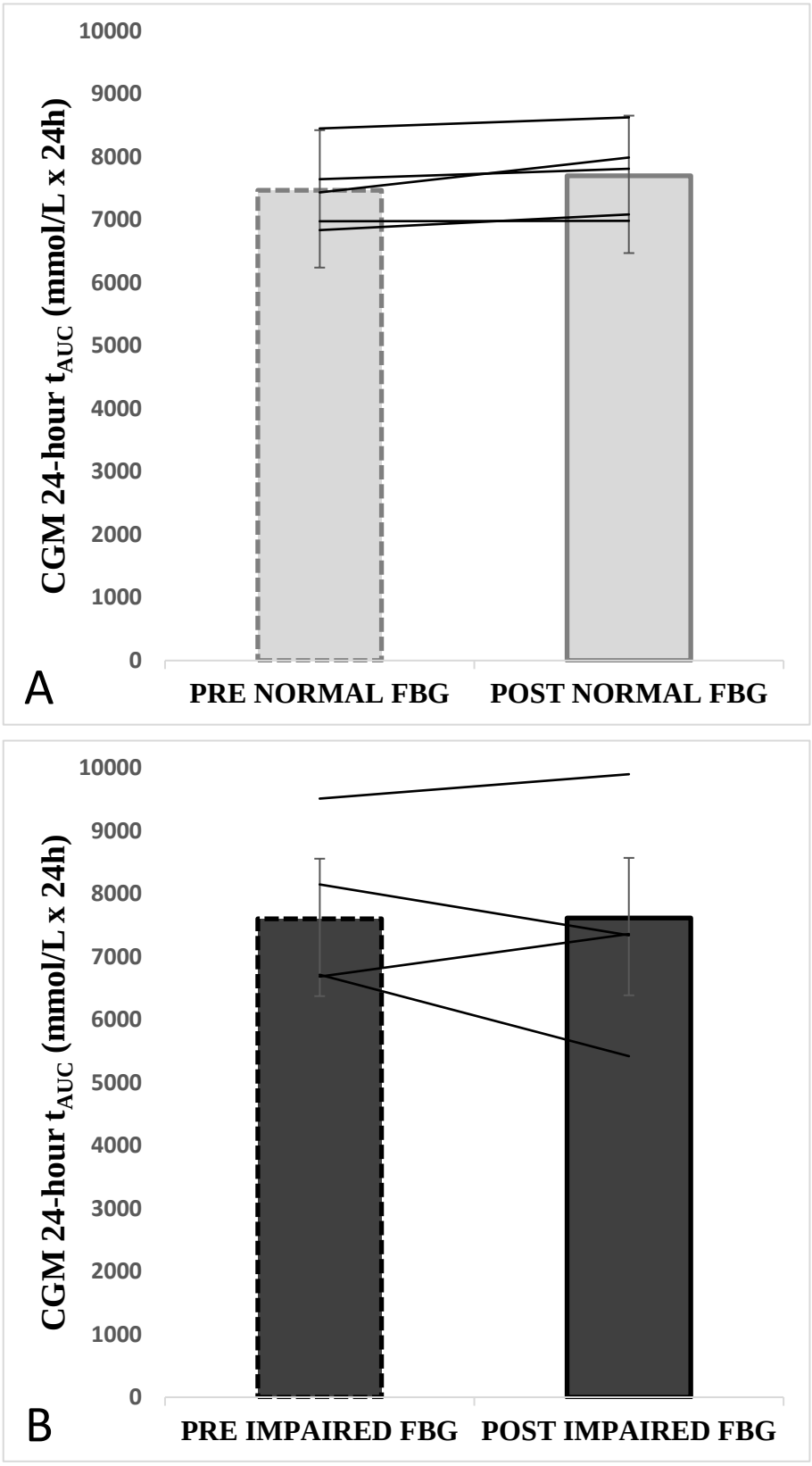

Figure 5.7 Pre- and post-intervention (week 0 vs week 7) continuous glucose monitor t_{AUC} over 24-hour period for (A) normal FBG (n = 5) and (B) impaired FBG (n = 4). Data presented as mean ± SD.

5.4.2.3 Ghrelin Response

Stage 1 (pre-intervention): Acute exercise-induced effect of supervised EFHIIT on post-prandial acyl and total ghrelin

There were no differences between groups for pre-intervention AG and TG t_{AUC} or at all individual timepoints ($p > 0.05$). (Figure 5.8 C&D). No significance for main effect of time*group [$F_{(2,16)} = 1.378$, $p = 0.280$] for AG and TG.

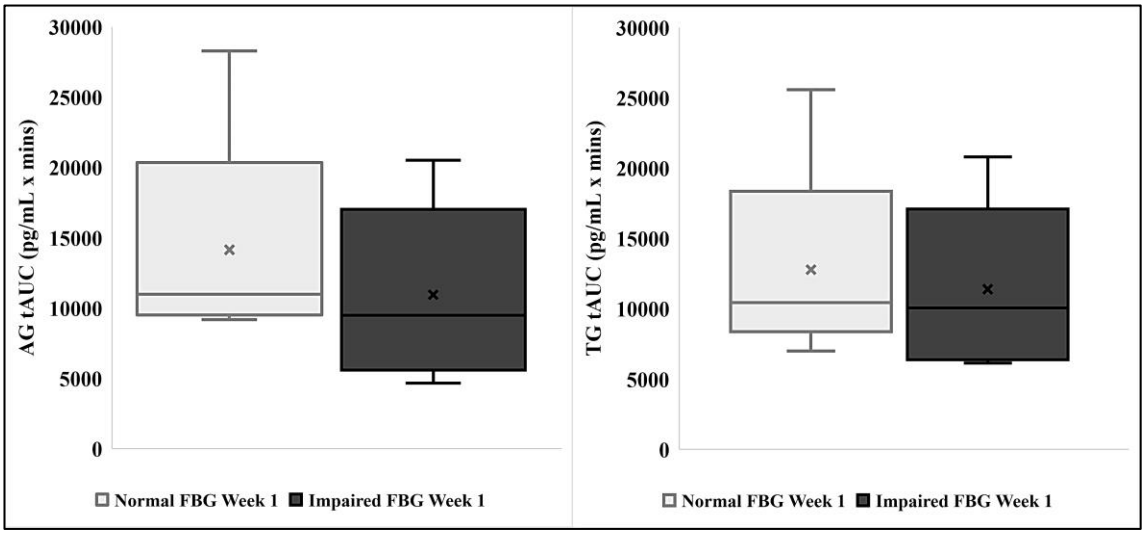

Figure 5.8 A&B: Post-prandial acyl ghrelin (AG) and total ghrelin (TG) t_{AUC} response prior and following an acute bout of EFHIIT in Week 1 in post-menopausal women with normal fasting blood glucose (FBG; N = 5), impaired FBG (N = 5). Blood samples for post-prandial AG and TG at Week 1 were obtained at timepoints thirty minutes after feeding during pre-exercise (0-EX), immediately post-exercise (EX), and thirty minutes post-exercise (30-EX). t_{AUC} was calculated for all timepoints for both groups. The line within the box plots represents the median, the lower and upper limits of the box are the 25th and 75th percentiles, and the error bars are the 10th and 90th percentiles.

In the normal FBG group, there were no simple effect for time for both AG [$F_{(2,8)}$ = 7.958, p = 0.147] and TG [$F_{(2,8)}$ = 2.003, p = 0.197] (Figure 5.8 A&B).

Conversely, the impaired FBG group exhibited significant simple effect for time in both AG [$F_{(2,8)}$ = 7.958, p = 0.013] and TG [$F_{(2,8)}$ = 7.994, p = 0.047]. There was a significant exercise-induced suppression in post-prandial AG and TG immediately after performing supervised acute EFHIIT (EX), denoted by a decrease of 58.4% and 29.0%, respectively (AG: 0-EX: 345.1 ± 200.4 pg/mL vs EX: 143.5 ± 118.4 pg/mL, p = 0.036; TG: 0-EX: 273.8 ± 151.8 pg/mL vs EX: 194.5 ± 43.2 pg/mL, p = 0.045) (Figure 5.8 A&B).

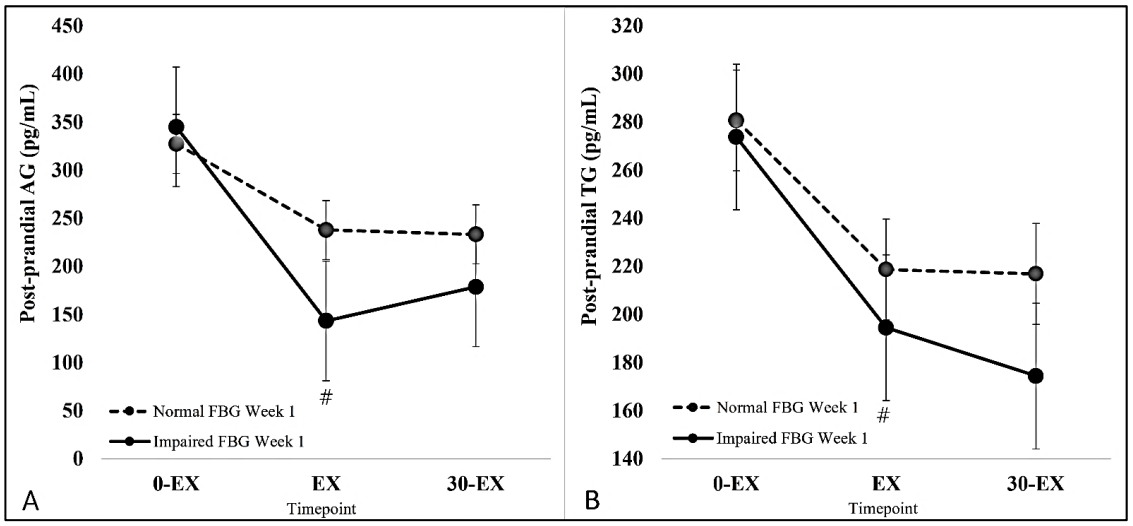

Figure 5.9 A&B: Post-prandial acyl ghrelin (AG) and total ghrelin (TG) response prior and following an acute bout of EFHIIT in Week in post-menopausal women with normal fasting blood glucose (FBG; N = 5), impaired FBG (N = 5). Blood samples for post-prandial AG and TG at Week 1 were obtained at timepoints thirty minutes after feeding during pre-exercise (0-EX), immediately post-exercise (EX), and thirty minutes post-exercise (30-EX). Error bars in the line graphs are presented as standard error of the mean. (# denotes p < 0.05 when compared to 0-EX).

Stage 2 (post-intervention): Chronic exercise-induced effect of HEFHIIT on post-prandial acyl and total ghrelin

AG and TG t_{AUC} did not differ between groups (Figure 5.9 A&B). There were no significant main effects for time*intervention*group for AG [$F_{(2,16)}$ = 0.792, p = 0.470] and TG [$F_{(2,16)}$ = 0.333, p = 0.721]. Pre- to post-intervention comparisons revealed that there was an increase in AG and TG t_{AUC} in both normal (AG: 14236.9 mins x pg/mL vs 19734.3 mins x pg/mL, p = 0.08; TG: 12778.4 mins x pg/mL vs 14256.0 mins x pg/mL, p = 0.345) and impaired FBG group (AG: 10942.5 mins x pg/mL vs 144550.7 mins x pg/mL, p = 0.094; TG: 11389.4 mins x pg/mL vs 13388.0 mins x pg/mL, p = 0.075), although this was not significant (Figure 5.10 A&B).

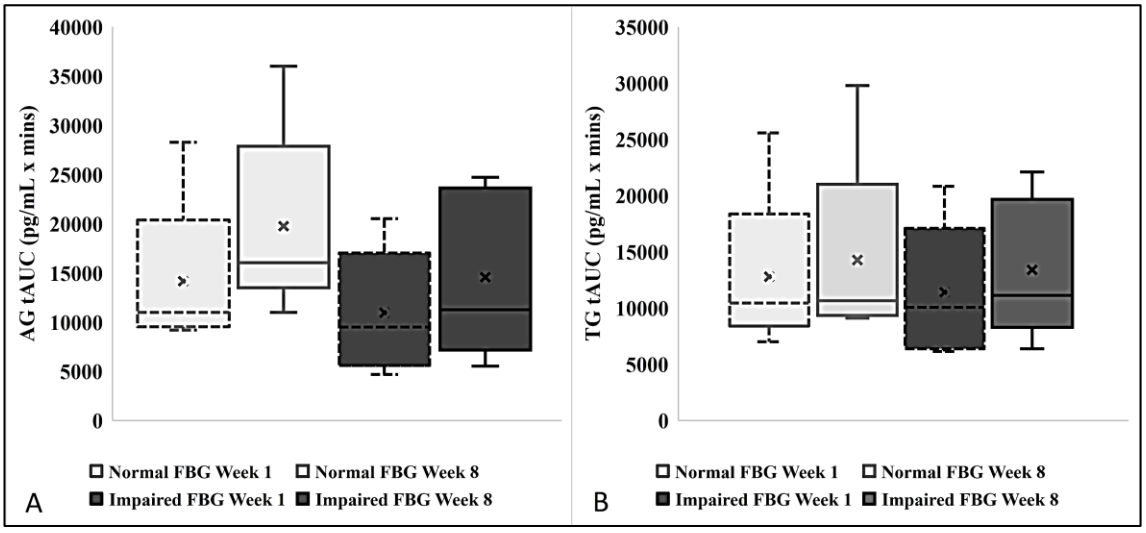

Figure 5.10 A&B: Post-prandial acyl ghrelin (AG) and total ghrelin (TG) t_{AUC} response prior and following an acute bout of EFHIIT in Week 1 vs Week 8 in post-menopausal women with normal fasting blood glucose (FBG; N = 5), impaired FBG (N = 5). Blood samples for post-prandial AG and TG at Week 1 were obtained at timepoints thirty minutes after feeding during pre-exercise (0-EX), immediately post-exercise (EX), and thirty minutes post-exercise (30-EX). t_{AUC} was calculated for all timepoints for both groups. The line within the box plots represents the median, the lower and upper limits of the box are the 25th and 75th percentiles, and the error bars are the 10th and 90th percentiles.

For the normal FBG group, there were no simple effect for time*intervention for AG [$F_{(2,8)}$ = 1.043, p = 0.396] and TG [$F_{(2,8)}$ = 0.110, p = 0.897].

Similarly, the impaired FBG group displayed no simple effect for time*intervention for AG [$F_{(2,8)}$ = 0.210, p = 0.815] and TG [$F_{(2,8)}$ = 1.127, p = 0.371]. Post-intervention, there was a blunted exercise-induced suppression of AG (58.4% vs 51.7%) and TG (22.1% vs 16.4%) in the impaired FBG group (Figure 5.11 B&D).

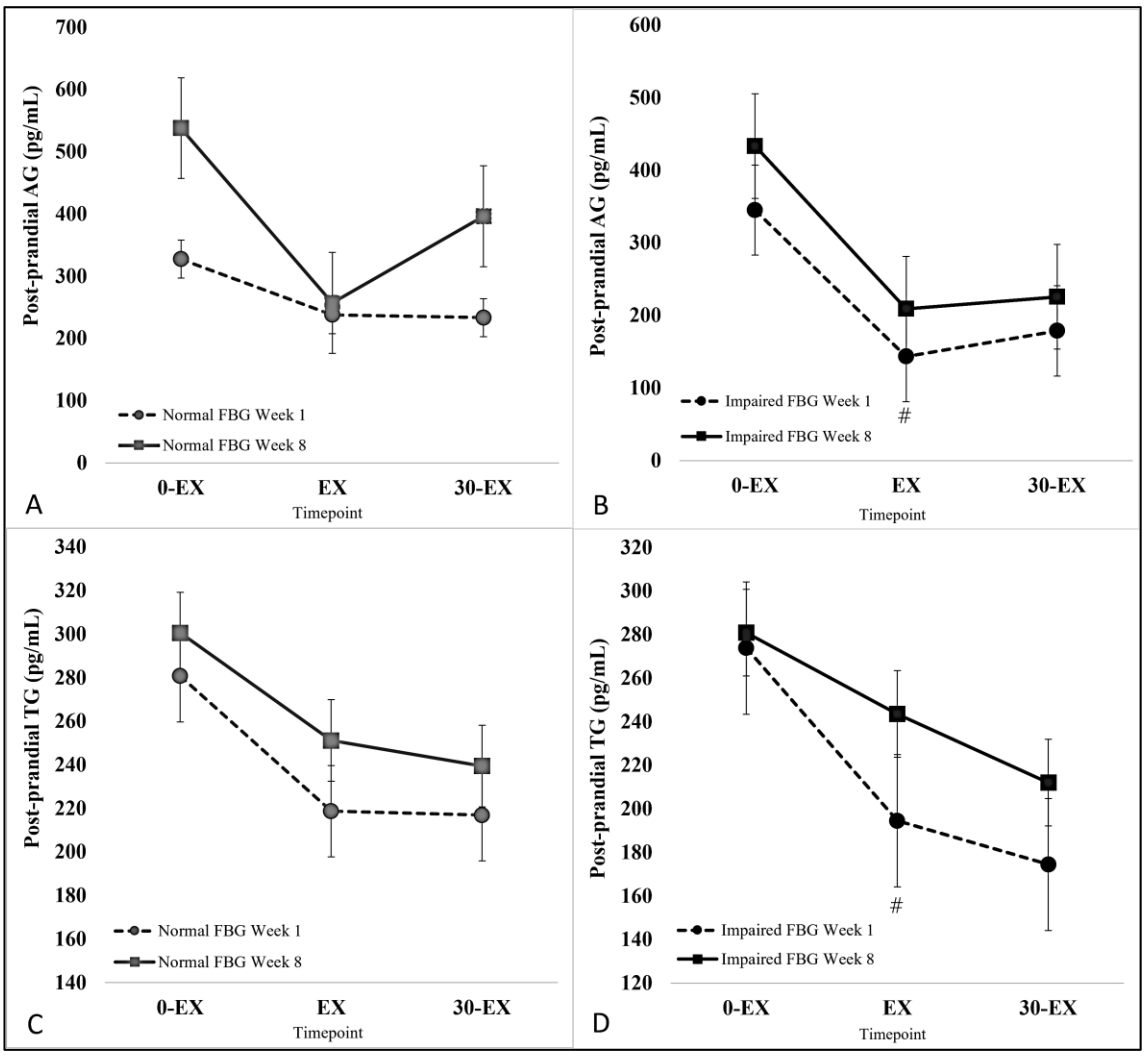

Figure 5.11 A-D: Post-prandial acyl ghrelin (AG) and total ghrelin (TG) response prior and following an acute bout of EFHIIT in Week 1 (●) vs Week 8 (■) in post-menopausal women with normal fasting blood glucose (FBG; N = 5), impaired FBG (N = 5). Blood samples for post-prandial AG and TG at Week 1 and Week 8 were obtained at timepoints thirty minutes after feeding during pre-exercise (0-EX), immediately post-exercise (EX), and thirty minutes post-exercise (30-EX). The line within the box plots represents the median, the lower and upper limits of the box are the 25th and 75th percentiles, and the error bars are the 10th and 90th percentiles. Error bars in the line graphs are presented as standard error of the mean. (# denotes p < 0.05 when compared to 0-EX).

5.4.2.4 Influence of Glycaemia on Chronic EFHIIT-induced alterations in Ghrelin and Impact on Cardiometabolic Risk Markers

The influence of baseline FBG on the relationship between chronic EFHIIT-induced changes in ghrelin and cardiometabolic risk markers were investigated. Linear regression found no relationship between baseline FBG on AG and TG changes in t_{AUC}, 0-EX, EX and 30-EX.

Bivariate correlations were employed to explore the influence of chronic EFHIIT induced changes in ghrelin and cardiometabolic risk markers in the stratified groups. In the impaired FBG group, change in AG t_{AUC} was positively correlated with change in DBP (r = 0 91, p = 0.034), and HbA1c (r = 0.96, p = 0.009). When adjusting for baseline FBG, the positive association was still significant between AG t_{AUC} and HbA1c (r = 0.99, p = 0.005), but not for DBP (r = 0.92, p = 0.08).

5.4.2.5 Habitual Activity and Compliance to Intervention

The average adherence rate to the intervention in both groups were 90% (21 out of 24 sessions). This did not differ between groups. Analysis of the GARMIN logged reports revealed that two participants in the normal FBG group and three participants with impaired FBG adhered 100% (24 sessions). There was an increase observed in step count in both groups when comparing Week 0 to Week 8 (Normal FBG: 5749 vs 7934 steps; Impaired FBG: 4776 vs 5596 steps, p > 0.05), although this was not significant between and within group.

5.5 Discussion

While other published studies have investigated laboratory, supervised HIIT interventions on cardiometabolic health in post-menopausal women, none involves the implementation of an unsupervised HEFHIIT. This easily implementable modality may aid to address the barriers to physical activity among this cohort (Tinker et al., 2017) and promote better cardiometabolic health. Furthermore, understanding the response of the orexigenic hormone ghrelin to acute and chronic exercise could aid a better understanding of an under-investigated metabolic pathway in mitigating metabolic dysfunction. While previous studies have evaluated the effects of chronic exposure of exercise on fasting ghrelin levels in post-menopausal women (Foster-Schubert et al., 2005; Mason et al., 2015; Steckling et al., 2019; Tremblay et al., 2019), none have investigated post-prandial AG or TG following acute exercise or an exercise intervention in this specific cohort. Consequently, comparing our results with previous investigations in response to both stimuli has been challenging.

In this novel study, the exercise modality of HEFHIIT supports our primary hypothesis, significantly showing improvements in SBP and DBP in the total cohort. This study is the first to investigate the feasibility of modulating post-prandial AG and TG profiles in response to acute and chronic exposure of EFHIIT in post-menopausal women. In agreement with our second hypothesis, we show that acute and chronic EFHIIT can mediate post-prandial AG and TG levels. Our findings demonstrated that in response to acute EFHIIT during both pre- and post-intervention, a significant decrease in post-prandial AG concentrations was exhibited. Eight weeks HEFHIIT significantly increased post-prandial AG and TG t_{AUC}. Additionally, in accord with the third hypothesis, participants with normal FBG and impaired FBG displayed opposing exercise-induced ghrelin responses acutely and chronically, as well as markers of cardiometabolic health exhibited through significant reductions in glycaemic variability and FBG levels, respectively.

5.5.1 Cardiometabolic Response to Exercise Intervention

In corroboration with the meta-analysis in Chapter 4, this study found significant improvements in at least one metabolic benefit with 8 weeks of HEFHIIT, exhibiting significant reductions in SBP (-6 mmHg) and DBP (-4 mmHg). There is a consensus from other meta-analyses on the efficacy of HIIT interventions (≥4 weeks) in reducing BP across all clinical and non-clinical populations in young, middle-aged (de Oliveira et al., 2023) and older adults (Carpes et al., 2022). The proposed mechanism has been theorised that through performing HIIE overtime, the frequent shear stress exerted on the vessel walls increases production of nitric oxide (NO), a potent vasodilator, thus improving endothelial function (Haram et al., 2009). No other cardiometabolic benefits were found in this study, potentially attributed by the short duration of the intervention and the lack of dietary monitoring. Previous meta-analysis on exercise training in pre- and post-menopausal women found HIIT protocols to be less effective in reducing abdominal fat mass and body weight when compared to pre-menopausal women, with HIIT interventions lasting ≤8 weeks to have no effect on body composition (Dupuit et al., 2020). However, the very small number of publications

involving post-menopausal women makes interpretation the findings to be inconclusive. Thus, in this present study, it cannot be ascertained if the absence of improvements in body weight and other cardiometabolic markers were contributed by overcompensation of energy intake (EI) from increased energy exertion or the duration of the study. Nevertheless, this study highlights the advantages of HEFHIIT without dietary restrictions in improving BP in post-menopausal women without clinical hypertension.

5.5.2 Acute Exercise-Induced Effects on Ghrelin

Exercise is frequently associated with a transient decrease in appetite known as exercise-induced anorexia (King et al., 1994). Suppression of exercise-induced AG have found to be positively correlated with exercise intensity (Anderson et al., 2021). In response to HIIE, the mechanism behind AG suppression could be underpinned by the redistribution of blood flow from the splanchnic area towards the skeletal muscles to support the high demand of exercise performed (Hazell et al., 2016). As ghrelin is predominantly secreted from the stomach, blood flow to the digestive system is reduced during exercise, thereby reducing the transport of ghrelin into circulation (Hazell et al., 2016). While it is known that obese individuals tend to exhibit a blunted post-prandial ghrelin response compared to normal weight individuals (Wang et al., 2022), limited knowledge exists regarding the impact of acute HIIE on post-prandial ghrelin in obese individuals. In response to a fasted, single bout of HIIE, studies conducted in young adults with obesity showed a transient suppression of AG (Holliday & Blannin, 2017) and TG (Matos et al., 2021) following exercise. Here, we show the suppressive effect of acute EFHIIT following an additional stimulus, i.e. feeding. It is established that circulating AG and TG levels decline post-prandially, regardless of weight status (Wang et al., 2022). In this study, circulating post-prandial AG and TG further declined following acute EFHIIT. Furthermore, this effect was replicated irrespective of habitual activity status (habitually sedentary or active i.e. pre-intervention or post-intervention, respectively), showing the feasibility of EFHIIT in suppressing ghrelin.

5.5.3 Chronic Exercise-Induced Effects on Ghrelin

Evidence show that obese individuals display lower baseline of circulating AG levels regardless of prandial state (Wang et al., 2022). Within this context, further investigation in the significant increase in post-intervention post-prandial AG levels and AG t_{AUC} indicate resemblance to that of non-obese individuals, implying a potential ghrelin-mediated restoration in metabolic and energy homeostasis (Wang et al., 2022). Individuals with obesity exhibit ghrelin resistance, evident through impaired fasting and post-prandial circulating ghrelin levels (Wang et al., 2022). This occurs due to impaired ghrelin secretion in the stomach, where ghrelin-secreting cells no longer respond effectively to the stimulatory actions of stimuli including nor-epinephrine or glucose (Uchida et al., 2014). AG is predominantly produced by the cells of the stomach, where the enzyme ghrelin O-acyltransferase (GOAT), is responsible for the acylation of ghrelin to AG. During exercise, blood flow is redistributed from the stomach to skeletal muscles in an intensity-dependent manner, thereby decreasing activity of GOAT and reducing circulating AG (Hazell et

al., 2016). This mechanism could explain that chronic exposure to HIIE over the eight weeks is restoring ghrelin sensitivity through repeated reductions in AG production, progressively increasing sensitivity to stimuli such as feeding. Although rate of gastric emptying was not measured in the present study, Davis *et al.* demonstrated that obese individuals that are regularly active exhibit increased rate of gastric emptying that accompanied higher postprandial AG t_{AUC} compared to non-physically active obese counterparts (Davis et al., 2020). Our findings mirror that of Davis *et al.,* providing evidence that eight weeks of HEFHIIT was sufficient in mediating resting post-prandial AG levels to that of those who are habitually active (Davis et al., 2020). This suggests that the significant increases in post-prandial ghrelin profiles in this study may be indicative of the restoration of ghrelin sensitivity evident by augmented rate of gastric emptying (Davis et al., 2020).

5.5.4 Chronic Exercise-Induced Ghrelin Levels and Cardiometabolic Risk Markers

Despite no other cardiometabolic benefits observed except for SBP and DBP, the attempt to explore the influence of chronic exercise-induced changes in ghrelin levels and cardiometabolic risk markers was warranted.

Restoration of ghrelin sensitivity through increases in AG and TG can be reinstated through weight loss as the body employs compensatory signals to restore body weight (Briggs et al., 2013). In addition to the significant increases in AG and TG post-intervention, it would be logical to assume weight loss. Interestingly, this study found an absence in weight loss or body composition changes post-intervention, mirroring previous studies (Church et al., 2009). As a regulator of energy metabolism, circulating ghrelin levels increase as a compensatory response to increased energy expenditure (EE), thereby stimulating fat storage and lipid accumulation to protect the body weight set point (Churm et al., 2017). This is observed in exercise programs, where despite efforts to counteract weight gain, the reality of weight loss is often lower than predicted in individuals with overweight and obesity (Thomas et al., 2012). Furthermore, studies show that AG treatment to human visceral adipocytes increase expression of lipogenic and adipogenic transcription factors (Rodríguez et al., 2009) and can increase white adipose tissue mass (Thompson et al., 2004). The absence of observed body fat percentage, visceral adiposity and lipid profile change in this study could be explained by the increase in AG and TG post-intervention. However, as nutritional intake throughout the study was not monitored, the absence of improvement in body weight, body composition and lipid profiles cannot be confirmed by means of augmented ghrelin levels or positive energy balance to compensate for increased EE.

Ghrelin plays a role in regulating the cardiovascular system through mediating sympathetic nerve activity and vasodilation through increasing cardiac output (Lund et al., 2023; Mao et al., 2016; Okumura et al., 2002). In this study, we did not find associations between baseline or change in AG and TG with BP. It is important to note that participants in this study had no previous clinical diagnosis of hypertension, although the average baseline SBP and DBP were 131 mmHg and 81 mmHg respectively. Whilst it has been found that ghrelin levels are lower in hypertensive

individuals and have an inverse correlation with BP (Pöykkö et al., 2003), ghrelin and BP are positively correlated in obese post-menopausal women with hypertension (Öner-Iyidoğan et al., T2009), highlighting the complexity of the relationship between ghrelin and BP. The limitation of statistical power prevents conclusive interpretations of ghrelin-mediated changes in cardiometabolic risk markers with HEFHIIT.

5.5.5 Influence of Glycaemia on Chronic Exercise-Induced Ghrelin Levels and Cardiometabolic Risk Markers

To better understand the relationship between ghrelin and glucose homeostasis in our cohort, participants were stratified for normal FBG (<6.1 mmol/L) and impaired FBG (≥6.1 mmol/L). Ghrelin and glucose homeostasis share an interrelationship (Chabot et al., 2014), thus warranting exploration into the influence of FBG status on exercise-induced ghrelin response. In agreement with the third hypothesis of this study, ghrelin response to acute EFHIIT during pre-intervention differed between the groups. A significant exercise-induced suppression of AG and TG was observed in the impaired FBG group that was absent in the normal FBG group. At post-intervention, the impaired FBG group exhibited a blunted exercise-induced suppression of AG and TG that was also absent in the normal FBG counterpart. This is in disagreement with Heiston *et al.* who found no change in postprandial suppression of AG in obese, middle-aged adults with prediabetes following a two-week HIIT intervention (Heiston et al., 2018). The duration of the intervention could explain the disparity between the results, suggesting that the 8-week HEFHIIT intervention could mediate ghrelin changes in post-menopausal women with impaired FBG.

While it is understood that ghrelin and glucose are interrelated, their relationship is complex and not fully understood. Thus, it is beyond the scope of this study to understand the mechanisms of the effects of varying FBG on ghrelin due to a knowledge gap that exists on the impact of impaired glycaemia on post-prandial ghrelin and exercise. Nevertheless, the relationship between exercise-induced changes in ghrelin and cardiometabolic response of varying FBG status was explored, revealing that HEFHIIT-induced increases in AG were positively associated with changes in DBP and HbA1c in the impaired FBG group. Interestingly, the relationship between changes in DBP and AG was mitigated after adjusting for baseline FBG but sustained for HbA1c. It is established that individuals with increasing MetS comorbidities exhibit lower ghrelin levels (Serra-Prat et al., 2009). Furthermore, acute AG administration has shown to downregulate insulin concentrations, thereby increasing FBG levels (Gauna et al., 2004). This has led to the theory that exercise-induced increase in AG may be unfavourable for individuals with impaired FBG, leading to the worsening of insulin sensitivity. However, the influence of glycaemia on chronic exercise-induced ghrelin levels and cardiometabolic risk markers in this study remains inconclusive due to its limitation in statistical power. Future studies should employ larger sample size to investigate the response of exercise-induced AG and DAG mediation to explore the effects of ghrelin isoforms and their relation to cardiometabolic response in varying FBG cohorts.

Participants with normal FBG exhibited notable reductions in glycaemic variability (GV) (-1.8 CV%). Contrastingly, this effect was not present in those with impaired FBG. GV plays a central role in assessing and monitoring glycaemic dysregulating over a period of time, with evidence linking to increased CVD risk (Liang et al., 2017). Studies in non-diabetic and diabetic patients that underwent sleeve gastrectomy exhibited significant improvements in GV with decreased TG levels post-surgery (Wang et al., 2021). Furthermore, there were positive correlations between the decrease in glucose metrics and the decrease in TG in the non-diabetic group (Wang et al., 2021). Indeed, this procedure removes the main site of ghrelin-producing cells, thus it was expected to observe the decreased plasma ghrelin levels following surgery. Paradoxically to the previous benefits of augmented ghrelin levels mentioned, these studies highlight the deleterious effect of ghrelin and its negative impacts on glycaemic homeostasis in a morbidly obese population. These studies could explain the significant improvement in GV seen in those with normal FBG that was absent in the impaired FBG of this study, as only the latter group exhibited increased AG and TG levels post-exercise after the intervention. It is noteworthy that despite the observed varying response to the intervention between groups in this study, no differences were found between groups when comparing AG and TG t_{AUC} or at all individual time points. This study evaluating the difference in glycaemic status on exercise-induced ghrelin profiles is underpowered, therefore more research is warranted to further investigate this effect.

5.5.6 Strengths and Limitations

This study has both strengths and limitations. It is important to emphasise that despite a small sample size, significant improvements in BP were observed. This is in accord with the findings of the meta-analysis in Chapter 4, highlighting the feasibility of HEFHIIT in improving this metabolic marker in non-hypertensive post-menopausal women. Additionally, a key strength was the effectiveness of eight weeks of unsupervised HEFHIIT in modulating ghrelin profile in post-menopausal women, without controlling for diet. However, there are some limitations that may impact the study's interpretations. This study's focus was to assess the feasibility of HEFHIIT in mediating ghrelin profile in an unsupervised manner, with instructions given to participants to consume nutrition *ad libitum.* Therefore, the effects of exercise-induced alterations in ghrelin profile on appetite, hunger and EI could not be ascertained. Moreover, it is acknowledged that this study lacks information on nutritional status and did not include a control group. There were also no fasted samples obtained for ghrelin, preventing the determination of the magnitude of response from fasting to post-prandial timepoints for AG and TG. There were also no post-intervention data for CRF measurement, limiting the analysis for the effects of the intervention on change in estimated aerobic capacity. Finally, due to the small sample size in both the normal and impaired FBG groups, interpretation should be made with caution. It is acknowledged that further studies with larger sample sizes are warranted to establish these findings.

5.6 Conclusion

In conclusion, this study underscores the feasibility of improving one aspect of cardiometabolic health and augmentation of post-prandial AG with 8 weeks of home, equipment-free HIIT (HEFHIIT) in post-menopausal women. There were significant improvements in systolic and diastolic blood pressure observed in the total cohort, strengthening the findings of the meta-analysis in Chapter 4. This study provides novel evidence on the increase in resting post-prandial AG, as well as post-prandial acyl (AG) and total ghrelin (TG) t_{AUC} profiles following 8 weeks of HEFHIIT. Upon stratifying groups for baseline fasting blood glucose (FBG), individuals with normal FBG significantly improved glycaemic variation and visceral adiposity, while those with impaired FBG exhibited significant reductions in FBG by 0.5 mmol/L and blood pressure. This further extends the metabolic health benefits of exercise of 8 weeks in post-menopausal women with different glycaemic status. There were no associations found between changes in ghrelin and cardiometabolic risk markers. Thus, warranting further research by amplifying the cardiometabolic response with the addition of dietary intervention to further understand ghrelin-mediated cardiometabolic health with lifestyle interventions.

Chapter 6 (GHREXD) – Investigating the cardiometabolic and ghrelin response to home, equipment free high-intensity training with or without the Mediterranean-style diet in post-menopausal women: a randomised controlled trial

6.1 Introduction

Despite efforts to counteract weight gain, the reality of weight loss from exercise programs is often lower than anticipated, particularly in individuals that are overweight or obese (Thomas et al., 2012). Indeed, exercise alone is effective in improving cardiometabolic health markers, as indicated in Chapters 4 and 5. However, a sustainable lifestyle intervention focused on healthy eating habits and consistent engagement in physical activity has been established to be the most efficacious approach to foster favourable body composition and mitigate the risk of obesity-related cardiometabolic complications (Donnelly et al., 2009). While Chapter 5 found significant improvements in SBP and DBP, as well as augmentation of AG levels with 8-week of HEFHIIT, no changes in weight loss or improvements in other cardiometabolic risk markers were found. The synergistic effect of combining diet with exercise may amplify the cardiometabolic response, thus aiding in the better understanding of ghrelin's role in mediating these risk factors.

Long-term lifestyle programs that incorporate both diet and exercise have reported greater effectiveness for weight loss than single component interventions alone (Johns et al., 2014). Meta-analyses of combined implementation of exercise and diet have indicated better cardiometabolic health outcomes than diet or exercise alone in overweight post-menopausal women (Cheng et al., 2018; Khalafi & Symonds, 2023). Despite its utility for initial weight loss, majority of these diets involve caloric restriction which can pose a challenge in terms of promoting adherence over the long-term (Oconnor et al., 2021), particularly among middle-aged individuals. Alternative dietary patterns that are more sustainable such as the Dietary Approach to Stop Hypertension (DASH) and the Nordic diet models have been designed as healthier substitutes to the traditional Western style diet and have been proven effective against markers of cardiometabolic risk (Massara et al., 2022; Vasei et al., 2022). However, unlike the Mediterranean diet (MedDiet), the DASH and the Nordic diet models lack a structured food pyramid, potentially serving as a hindrance to facilitating long-term adherence and sustainability in weight loss efforts (Dayi & Ozgoren, 2022).

6.1.1 Mediterranean Diet and Cardiometabolic Health

Since the 1960s, researchers have observed the positive impact of dietary patterns in Mediterranean countries on health, attributable to its high abundance in anti-inflammatory and antioxidant nutrients (Sofi et al., 2010). The MedDiet, while exhibiting variations across countries, is generally characterised by:

1) high consumption of vegetables, fruits, legumes, nuts, fish, and monounsaturated fatty acids (MUFAs);
2) moderate consumption of dairy products, meats, and wine;
3) limited consumption of saturated fatty acids (SFAs),

all of which are readily available components in any European region (Zupo et al., 2023). A depiction of the MedDiet pyramid is depicted in Figure 6.1. Mortality and morbidity rates are

notably lower in Mediterranean countries than those in Northern European countries and the United States (Menotti & Puddu, 2015). Recently, the WHO (Renzella et al., 2018) alongside other scientific experts (Donnelly et al., 2009) have strongly advocated for the adoption of the MedDiet as a preventative measure against chronic diseases. Supporting this, a recent meta-analysis highlighted the MedDiet as the singular dietary strategy associated with a reduced risk of major cardiovascular events and all-cause mortality (Doundoulakis et al., 2023). An umbrella review of meta-analyses further reported the superior effectiveness of the MedDiet in improving body weight and WC compared to controlled diets (Dinu et al., 2018). Additional studies have also revealed that high adherence to the MedDiet is associated with numerous other cardiometabolic health benefits including BP (Bakaloudi,et al., 2021), as well as targeting hallmarks of ageing (Shannon et al., 2021) through reducing the risk of age-related diseases such as T2D (Sarsangi et al., 2022), MetS (Bakaloudi et al., 2021) and CVD (Rosato et al., 2019). Furthermore, the 2020 European Menopause and Andropause Society (EMAS) position statement have summarised the existing evidence on MedDiet and menopausal health, highlighting the associations between short-term and long-term high adherence with reduced cardiovascular risk and all-cause mortality in menopausal and post-menopausal women (Cano et al., 2020).

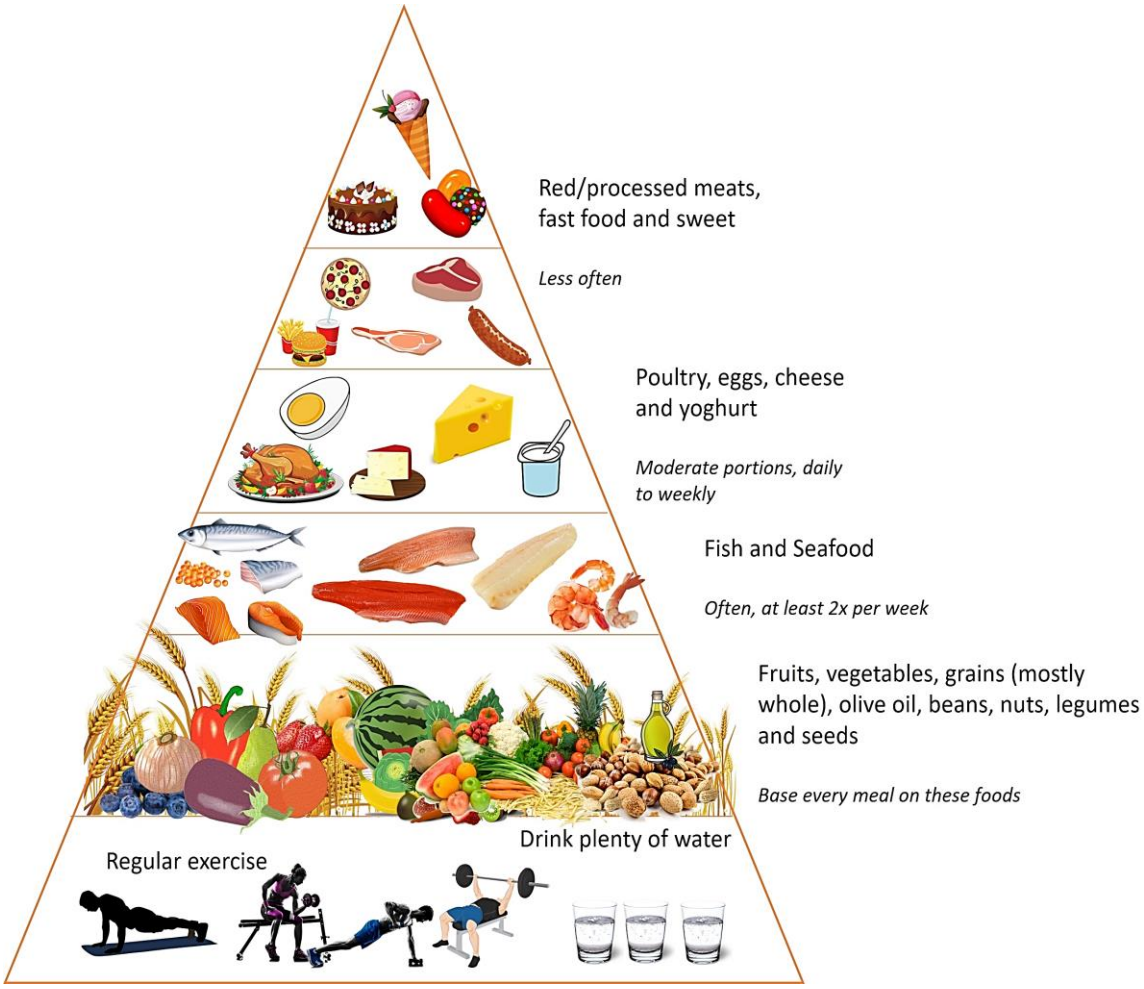

Figure 6.1 The Mediterranean diet pyramid.

6.1.2 Ghrelin Response to Lifestyle Interventions and Markers of Cardiometabolic Health

There is a consensus from a small body of literature on the increase in ghrelin levels associated with weight loss interventions. In overweight and obese post-menopausal women, greater increases in TG were associated with greater increases in weight loss that followed 12 months of dietary and/or exercise intervention (Mason et al., 2015). Similarly, this was mirrored in obese middle-aged adults, where weight loss from a 16-week caloric-restrictive diet was associated with increased DAG (Barazzoni et al., 2021). To date, only one study has investigated the effects of the MedDiet with exercise on ghrelin levels (Tsaban et al., 2022). In agreement with the previously mentioned studies, 18 months of the intervention resulted in increases in TG that accompanied weight loss in overweight/obese middle-aged adults. Interestingly, elevations in TG were associated with improvements in insulin sensitivity and VAT reductions, beyond weight loss (Tsaban et al., 2022). This relationship is also supported by previous studies that reported increases in ghrelin levels and improved insulin sensitivity resulting from weight loss (Barazzoni et al., 2021; Mason et al., 2015), supporting the notion for the association between lifestyle intervention-induced ghrelin increases and improvements in cardiometabolic health.

6.2 Aims

Studies to date have investigated the effects of a calorie restrictive diet with or without exercise on ghrelin levels. However, none have explored HIIT with or without MedDiet without caloric restriction in post-menopausal women. The benefits of HEFHIIT on improving at least one cardiometabolic risk marker is reported in Chapter 5 as well as evidencing the augmentation of post-prandial resting AG, as well as AG and TG t_{AUC} following the intervention, independent of weight loss. The synergistic cardiometabolic effects of MedDiet and HEFHIIT may elicit better cardiometabolic health outcomes, thus offering a better insight into the relationship between lifestyle-intervention induced changes in ghrelin and cardiometabolic health.

This study aims to:

1) Establish the effects of 8-week HEFHIIT with/without MedDiet on cardiometabolic markers.
2) Determine the effects of 8-week HEFHIIT with/without MedDiet on AG and DAG response.
3) Explore the relationship between HEFHIIT with/without MedDiet-induced changes in ghrelin and cardiometabolic risk markers.

Therefore, it is hypothesised that:

1) 8 weeks of HEFHIIT with the MedDiet will improve cardiometabolic risk markers compared to HEFHIIT only and/or control.
2) AG and DAG response differ between HEFHIIT with or without the MedDiet.
3) Changes in ghrelin are associated with changes in cardiometabolic risk markers.

The primary endpoint:

1) Between-group changes in cardiometabolic risk markers for control and HEFHIIT with/without MedDiet.

The secondary endpoints:

1) Changes in cardiometabolic risk markers following HEFHIIT with/without MedDiet compared to baseline.
2) Changes in fasted AG and DAG response following HEFHIIT with/without MedDiet compared to baseline.
3) Changes in fasted AG and DAG response between HEFHIIT with/without MedDiet.
4) Association between HEFHIIT with/without MedDiet-induced changes in ghrelin with changes in cardiometabolic risk markers.
5) Association between changes in leptin and adiponectin with cardiometabolic risk markers
6) Adherence and compliancy to the interventions.
7) Changes in energy balance (nutrition and energy expenditure) compared to baseline.

6.3 Materials and Methods

6.3.1 Ethical Approval

This study was reviewed and was given favourable ethical opinion for conduct by the Hampstead Research Ethics Committee (22/LO/0301; IRAS ID: 314505) (Appendix 3). This study was conducted in accordance with the Declaration of Helsinki and registered with ClinicalTrials.gov (NCT05417698). All participants have given written and informed consent prior to participation.

6.3.2 Participants

A total of 126 potential participants expressed interest and were sent a participant information sheet (PIS) (Appendix 3) denoting the full description of the study. The PIS also contained the inclusion criteria:

1) post-menopausal (defined by cessation of menstruation for at least 12 consecutive month) (Davis et al., 2015)
2) aged 45 – 65 years, BMI >25 kg/m^2
3) no known diseases
4) generally well to exercise and physically inactive (defined by IPAQ score category I and not engaged in at least 60 min/week of structured exercise in previous 6 months).

During pre-screening, 83 potential participants were excluded; did not meet the inclusion criteria (n = 43), declined/lost contact (n = 32), unable to commit to the study due to lack of time (n = 8). In total, 43 potential participants were then invited on study site for screening. Upon screening, 8 participants did not meet inclusion criteria: low BMI (n = 5), on blood pressure tablet (n = 2), physically active (n = 1). A total of 35 participants passed screening and were subsequently randomised and allocated to their group assignment. A CONSORT flow diagram depicting recruitment of study participants is denoted in Figure 6.2.

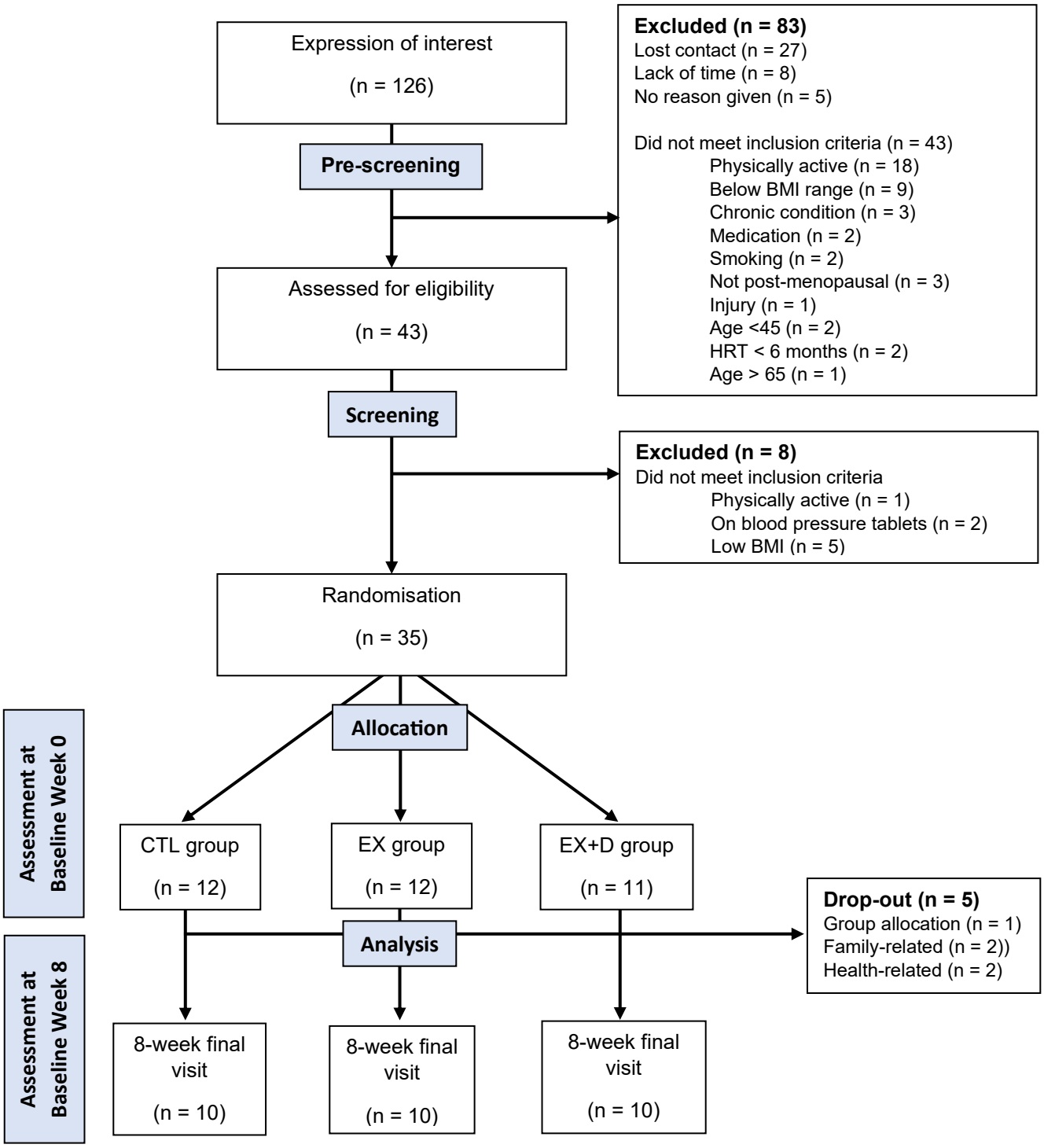

Figure 6.2 CONSORT flow diagram of participant recruitment and group allocation.

6.3.3 Study Design and Randomisation

This was a three-arm randomised-controlled study investigating the cardiometabolic and ghrelin response with an 8-week HEFHIIT intervention with/without the MedDiet. Conducted by the principal investigator (R.C), computerised random assignment was used to randomly assign eligible participants to: 1) control (CTL; n = 12); exercise only (EX; n = 12); exercise and MedDiet (EX+D; n = 11). This procedure involved the participants opening identical sealed opaque envelopes in sequential order containing their group allocation at the end of the screening visit. The allocation was concealed from the researcher in a concealed enveloped and was revealed to the enrolled participant after being deemed eligible. All eligible participants reported back to the laboratory for two separate visits in week 1 and week 8 respectively to complete baseline and post-intervention assessments. Participants in CTL were instructed to maintain their usual level of activity and diet during the 8-week study period. In EX and EX+D groups, participants were instructed to exercise for 20-minutes, three times a week, and those in the latter adhered to a MedDiet. No dietary prescriptions were provided for participants in the CTL and EX groups. There were no caloric restrictions, and all participants recorded their daily food diary on an app (MyFitnessPal) across all weeks.

6.3.4 Experimental Protocol

6.3.4.1 Screening Visit

A schematic of the screening visits, laboratory visits 1 and 2 is depicted below (Figure 6.3). In week 0, participants attended a screening visit for their eligibility to be assessed. Upon arrival at the laboratory following an overnight fast (≥12 hours) and having abstained from alcohol in the previous 24 hours, participants gave informed consent, completed a menopause questionnaire and an IPAQ (Appendix 3). Next, anthropometrics and an ECG were taken to assess eligibility. Once eligibility was ascertained, the participant's group allocation was revealed, and they were provided with an accelerometer (Garmin Forerunner 35, Garmin Ltd, United States). Questionnaires and body composition were collected as elaborated in Section 3.4.

6.3.4.2 Laboratory Visits 1 and 2

In weeks 1 and 8, participants arrived following an overnight fast from 2000h (≥12 hours) and having abstained from alcohol in the previous 24 hours. Participants had their anthropometrics, body composition and seated BP taken, followed by a whole-body scan via a dual-energy X-ray absorptiometry (DEXA) (Section 3.4.2) to assess body composition including total body fat percentage and visceral adiposity. A 16mL fasting blood sample was obtained from the antecubital vein into EDTA K_2 BD Vacutainer® tubes. Participants performed the Chester step test in both visits to assess their estimated cardiorespiratory fitness (CRF) (Section 3.4.4) and completed the IPAQ to assess any change in habitual physical activity in laboratory visit 2.

Participants in the EX and EX+D groups underwent a comprehensive briefing regarding the education of equipment-free HIIT (EFHIIT), involving a range of exercise types and form, target

heart rate and RPE with the researcher. Participants performed a supervised familiarisation session of an acute bout of equipment-free HIIT at ≥80% of maximum heart rate (HRmax) and corresponding to ≥18 Borg RPE scale (Borg, 1982) for 20-minutes. Adherence was measures and assessed as elaborated in section 3.5.

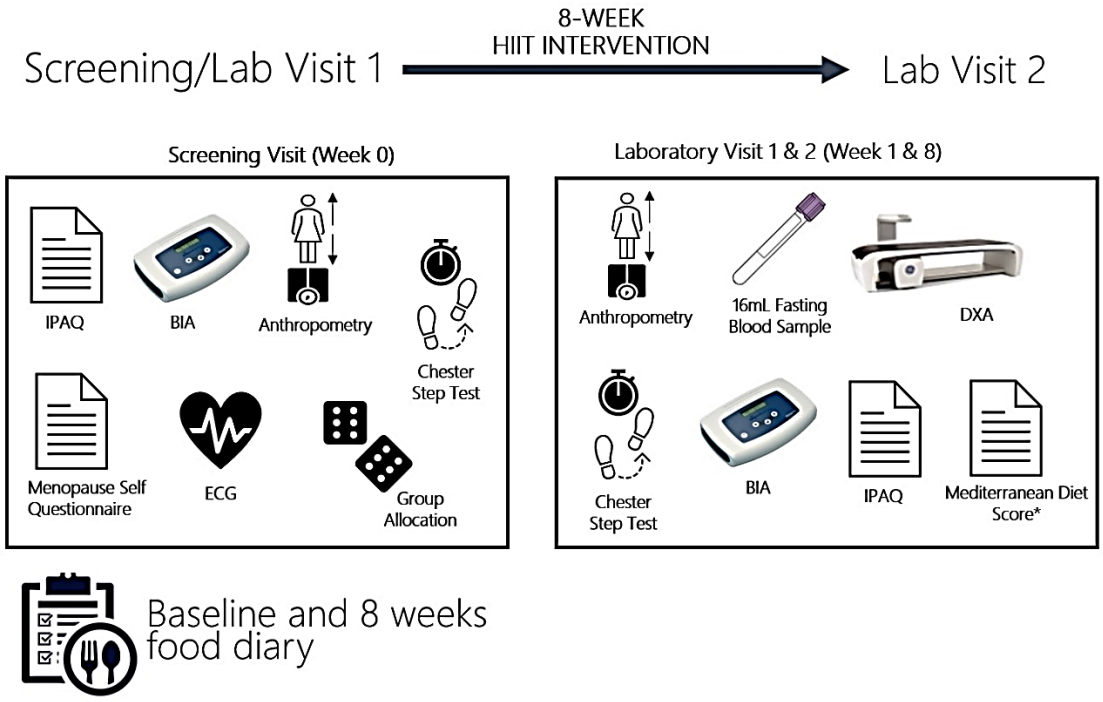

Figure 6.3 Schematic of the screening visit, laboratory visits 1 and 2. IPAQ: International Physical Activity Questionnaire; BIA: bioelectrical impedance analysis; ECG: electrocardiogram; DEXA: dual energy X-ray absorptiometry. *depicts completion by participants in EX+D only.

6.3.5 Mediterranean-Style diet and Measurement of Adherence

In addition to the tri-weekly exercises, participants in EX+D were instructed to adhere to a MedDiet. This involved a diet of meals mainly based around an abundance of plant-based foods, legumes and whole grains, moderate consumption of cheese, yoghurt, fish, poultry, and low consumption of red/processed meat and alcohol with a focus of utilising olive-oil as the main source of fat.

To determine the degree of adherence and changes to the MedDiet in the EX+D group, participants completed a specific, short 14-item questionnaire at the end of each week (from baseline to week 8) (Table 6.1). This questionnaire was the MedDiet adherence (MEDAS) score was used by the Mediterranean Diet Prevention Group (PREDIMED) (Martínez-González et al., 2002). A value of '1' and '0' were assigned to each item if they were achieved or not achieved respectively. From the sum of values, the degree of adherence was determined. Out of a total score of 14, optimal adherence to the MedDiet was considered high for scores ≥9, and low for scores <9 (León-Muñoz et al., 2012). To accommodate for participants that were vegetarian (n =

2) and did not consume alcohol (n = 2), all scores were adjusted as percentages (low adherence < 65%; high adherence ≥ 65%). Specifically, participants that were vegetarian had a score out of 11 (excluding question item 5, 10 and 13), and participants that did not consume alcohol had a score out of 13 (excluding question item 8).

To investigate the effect of the diet in EX+D, MEDAS scores of all 14-item from MyFitnessPal (FMP) logs for all three groups were obtained across all weeks.

Table 6.1 Validated 14-item Mediterranean diet adherence score (MEDAS) tool adapted from the PREDIMED study (Martínez-González et al., 2002)**.**

	Yes	No
1. Is olive oil the main culinary fat used?		
2. Are ≥ 4 tablespoons of olive oil used each day?		
3. Are ≥ 2 servings (of 200g each) of vegetables eaten each day?		
4. Are ≥ 3 servings of fruit (of 80g each) eaten each day?		
5. Is < 1 serving (100-150g) of red meat/ hamburgers/ other meat products eaten each day?		
6. Is < 1 serving (12g) of butter, margarine or cream eaten each day?		
7. Is < 1 serving (330ml) of sweet or sugar sweetened carbonated beverages consumed each day?		
8. Are ≥ 3 glasses (of 125ml) of wine consumed each week?		
9. Are ≥ 3 servings (of 150g) of legumes consumed each week?		
10. Are ≥ 3 servings of fish (100-150g) or seafood (200g) eaten each week?		
11. Is < 3 servings of commercial sweets/pastries eaten each week?		
12. Is ≥ 1 serving (of 30g) of nuts consumed each week?		
13. Is chicken, turkey or rabbit routinely eaten instead of veal, pork, hamburger or sausage?		
14. Are pasta, vegetable or rice dishes flavoured with garlic, tomato, leek or onion eaten ≥ twice a week?		
TOTAL SCORE (total number of 'yes' answers)		

6.3.6 Biochemical Analysis

Venous fasting blood samples were obtained from the antecubital vein into K_2EDTA BD Vacutainer® tubes to yield plasma with the exception of HbA1c, which was analysed with whole blood. Plasma samples were used to measure fasting HDL, TC, TRG and FBG as described in section 3.7. LDL was quantified as described in section 3.7.3.

Total insulin levels were quantified using fasting EDTA plasma samples with commercial, human-specific ELISA kits (Invitrogen: Cat# KAQ1251). The intra-assay coefficient of variation was 7.9%.

Whole blood (1992 µL) was aspirated into a microtube containing a protease inhibitor – AESBF (Millipore-Merck, Burlington, MA, USA) to a final concentration of 2 g/mL to preserve the octanoyl moiety of ghrelin in the blood. Fasting whole blood samples were immediately placed on ice following blood draw and processed within 15-minutes of collection. Blood tubes were centrifuged at 5,000 rpm for 5 minutes at 4°C to yield plasma. Aliquots of plasma was stored at -80°C until assayed. Commercial human-specific ELISA kits were used to quantify leptin and adiponectin (R&D systems; DY398-05 and DY1065-05 respectively), as well as AG and DAG (Bertin Bioreagent; Cat# A05306 and Cat# A05306 respectively). The intra-assay coefficient of variations were 9.8% for leptin, 1.4% for adiponectin, 2.6% for AG and 2.9% for DAG.

6.3.7 Sample Size Calculation

Based on the outcome determined from our previous feasibility study in Chapter 4, *a priori* sample size calculation was completed using GPower 3.1. Using a large effect size from Chapter 4 exploring AG mediated alteration with HEFHIIT, the proposed sample size to produce efficient power (80%) would be a minimal sample size of 10 individuals per group to complete the intervention of which this study achieved. Recruitment was set at a minimum of 38 participants accounting for 20% drop-out to detect difference in AG mediated alterations with HEFHIIT with 80% statistical power. Post-hoc power calculation was employed for one-way ANOVA intervention effect for AG and DAG, revealing effect size (Cohen's f) of 0.61 and 0.64.

6.3.8 Statistical Analysis

All statistical analysis of raw data was conducted using SPSS and Microsoft Excel. A p-value of <0.05 was considered statistically significant for all tests. Categorical data are represented by numbers (n) and percentage (%) and analysed using Pearson chi squared test. Normality was examined using Shapiro-Wilk test. For the primary analysis of between group variables, one-way analysis of covariance (ANCOVA) with baseline values as covariates were used for treatment effects between groups, with the Bonferroni test used for *post-hoc* analysis. For secondary analysis of within-group variables (baseline vs post-intervention), comparisons were performed using the student paired t-test for parametric variables, or Wilcoxon signed-rank test for non-parametric variables. Comparisons for between-group variables, one-way analysis of variance (ANOVA) was utilised to compare the baseline measures for normally distributed data and Kruskal-Wallis test for not normally distributed data. The relationship between intervention

changes in AG and DAG with cardiometabolic risk markers was analysed with linger regression analysis. MEDAS scores from MyFitnessPal instead of self-reported MEDAS scores were used for correlation analyses. Using FBG and insulin values, the Homeostasis Model Assessment 2 (HOMA2) Calculator version 2.2 (University of Oxford, Oxford, UK) was used to estimate β-cell function (HOMA2-%B), insulin sensitivity (HOMA2-%S) and IR (HOMA2-IR). QRISK3 scores were estimated using the QRISK3 algorithm (QRISK3 version 2018.0). Visceral adiposity index (VAI) was calculated with the following equation:

$$\frac{Waist\ circumference\ (cm)}{36.58 + (1.89\ \times\ BMI)} \times \frac{TG\ (mmol/L}{0.81} \times \frac{1.52}{HDL\ (mmol/L)}$$

6.4 Results

6.4.1 Baseline Characteristics

Table 6.2 displays the baseline characteristic of all study participants. In the total cohort (n = 30), participants mean age were 56.7 years. There were no differences in baseline characteristics among the three study arms ($p > 0.05$) except for weight ($F_{(2, 29)} = 4.03$, $p = 0.029$) and hip circumference (HC) ($F_{(2, 29)} = 4.02$, $p = 0.03$), where the CTL group had significantly higher weight and HC scores ($p < 0.05$).

Table 6.2 Baseline physical characteristics of total cohort.

General Characteristics	Total (n = 30)	CTL (n = 10)	EX only (n = 10)	EX+D (n = 10)	*P*
Age	56.7 ± 3.9	54.7 ± 3.3	57.7 ± 4.8	57.4 ± 3.3	0.25
Years of Menopause (years)	6.5 (3.0, 10.0)	4 (2, 7.5)	8.1 ± 3.3	6.5 (3, 11)	0.43
Weight (kg)	81.5 (68.5, 88.6)	93.9 ± 24.0	77.1 ± 9.8	75.7 ± 10.5	**0.029**
Height (m)	1.64 ± 0.06	1.67 ± 0.07	1.61 ± 0.05	1.64 ± 0.05	0.14
BMI (kg/m^2)	28.8 (26.4, 32.7)	31.5 (27.6, 42.0)	29.7 ± 3.5	28.2 ± 3.4	0.05
Waist (cm)	92.8 ± 10.8	92.0 (76.3, 120.3)	91.9 ± 8.4	88.7 ± 9.4	0.16
Hip (cm)	114.1 ± 13.3	124.3 ± 16.4	109.2 ± 6.1	110.1 ± 9.2	**0.03**
Waist to hip ratio (WHR)	0.81 ± 0.06	0.80 ± 0.07	0.84 ± 0.06	0.81 ± 0.05	0.33
SBP (mmHg)	124.1 ± 14.2	118 ± 8	126 ± 8	129 ± 21	0.24
DBP (mmHg)	84.3 ± 7.5	83 ± 6	86 ± 6	85 ± 10	0.55
Estimated Aerobic Capacity (mlO_2/kg/min)	29.9 ± 5.7	28.6 ± 4.6	30.1 ± 6.0	30.0 ± 4.8	0.47

Data presented as mean ± SD or median (IQR). BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

6.4.2 Intervention Effects on Body Composition and Cardiometabolic Risk Markers

6.4.2.1 Body Composition

When compared to CTL, baseline weight ($p = 0.03$), HC ($p = 0.03$), body fat mass ($p = 0.03$) and lean mass ($p = 0.04$) were significantly lower in the EX and EX+D groups (Table 6.3). There were no significant differences in baseline BMI, WC and body fat percentage ($p > 0.05$). There was a significant main effect for time for all body composition variables (Figure 6.4).

Compared to CTL, weight ($p < 0.05$), BMI ($p < 0.01$) and WC ($p < 0.05$) significantly decreased in EX and EX+D. There were also significant differences for HC ($p = 0.01$) and body fat mass ($p = 0.01$) between CTL vs EX+D, and lean mass ($p = 0.02$) and VAT mass ($p = 0.007$) between CTL vs EX.

Notably in CTL, there were significant increases in weight (+1.2 kg, p = 0.003) and BMI (0.4 kg/m^2, p = 0.009). In EX, there were significant decreases in WC (-2.1 cm, p = 0.02) and VAT mass (-327.5 g, p = 0.002). In EX+D, there were significant improvements in all body composition variables (weight: -1.5 kg, p = 0.005; BMI: -0.6 kg/m2, p = 0.004; WC: -1.8 cm, p = 0.005; HC: -2.6 cm, p = 0.03; body fat percentage: -2.4%, p = 0.01; body fat mass: -2.4 kg, p = 0.004; lean mass: 0.9 kg, p = 0.049; VAT mass: -114.1 g, p = 0.01).

Table 6.3 Baseline and post-intervention body composition variables. Data are presented as mean ± SD for normally distributed data. BMI: body mass index; VAT: visceral adipose tissue; $^{\$}$ denotes significance between EX and EX+D, and $^{\#}$denotes significance compared to CTL. $^{\#}$ p < 0.05; $^{\#\#}$ p < 0.01; $^{\#\#\#}$ p < 0.001. G: group effect.

Variable		**Groups**			***P* (G)**
		CTL (n = 10)	**EX (n = 10)**	**EX+D (n = 10)**	
Weight (kg)	**Pre**	93.9 ± 24.0	77.1 ± 9.8$^{\#}$	75.7 ± 10.5$^{\#}$	**<0.001**
	Post	95.0 ± 24.4	76.4 ± 10.2$^{\#\#}$	74.2 ± 9.9$^{\#\#\#}$	
	Δ	1.2 ± 0.8	-0.6 ± 1.2	-1.5 ± 1.3	
	P	**0.003**	0.2	**0.005**	
BMI (kg/m^2)	**Pre**	33.5 ± 6.8	29.7 ± 3.5	28.2 ± 3.4	**<0.001**
	Post	33.9 ± 6.9	29.5 ± 3.6$^{\#\#}$	27.7 ± 3.1$^{\#\#\#}$	
	Δ	0.4 ± 0.3	-0.3 ± 0.5	-0.6 ± 0.5	
	P	**0.009**	0.2	**0.004**	
Waist circumference (cm)	**Pre**	99.1 ± 12.7	91.9 ± 8.4	88.7 ± 9.4	**0.024**
	Post	98.7 ± 13.1	89.9 ± 9.6$^{\#\#}$	86.9 ± 9.8$^{\#}$	
	Δ	-0.4 ± 2.6	-2.1 ± 2.2	-1.8 ± 1.5	
	P	0.6	**0.02**	**0.005**	
Hip circumference (cm)	**Pre**	124.3 ± 16.4	109.2 ± 6.1	110.1 ± 9.2	**0.035**
	Post	122.9 ± 17.7	108.8 ± 7.8	107.5 ± 8.5$^{\#}$	
	Δ	-1.3 ± 5.0	-0.5 ± 2.5	-2.6 ± 3.2	
	P	0.3	0.6	**0.03**	
Body fat (%)	**Pre**	46.3 ± 4.7	44.1 ± 3.7	42.1 ± 4.1$^{\#}$	**0.041**
	Post	45.6 ± 5.3	44.2 ± 4.4	39.7 ± 4.6$^{\$}$	
	Δ	-0.8 ± 1.4	0.1 ± 1.7	-2.4 ± 2.4	
	P	0.1	0.9	**0.013**	
Lean Mass (kg)	**Pre**	49.7 ± 9.1	42.2 ± 4.5$^{\#}$	43.8 ± 5.0$^{\#}$	**0.035**
	Post	50.7 ± 8.9	42.5 ± 4.3$^{\#}$	44.5 ± 4.3	
	Δ	1.0 ± 1.5	-0.3 ± 1.5	0.9 ± 1.3	
	P	0.06	0.6	**0.049**	
VAT (g)	**Pre**	1388.8 ± 802.7	1177.9 ± 383.1	768.5 ± 361.7	**0.016**
	Post	1312.2 ± 769.3	850.4 ± 376.2$^{\#\#}$	654.4 ± 375.0	
	Δ	-76.7 ± 70.2	-327.5 ± 233.8	-114.1 ± 74.2	
	P	0.28	**0.002**	**0.01**	

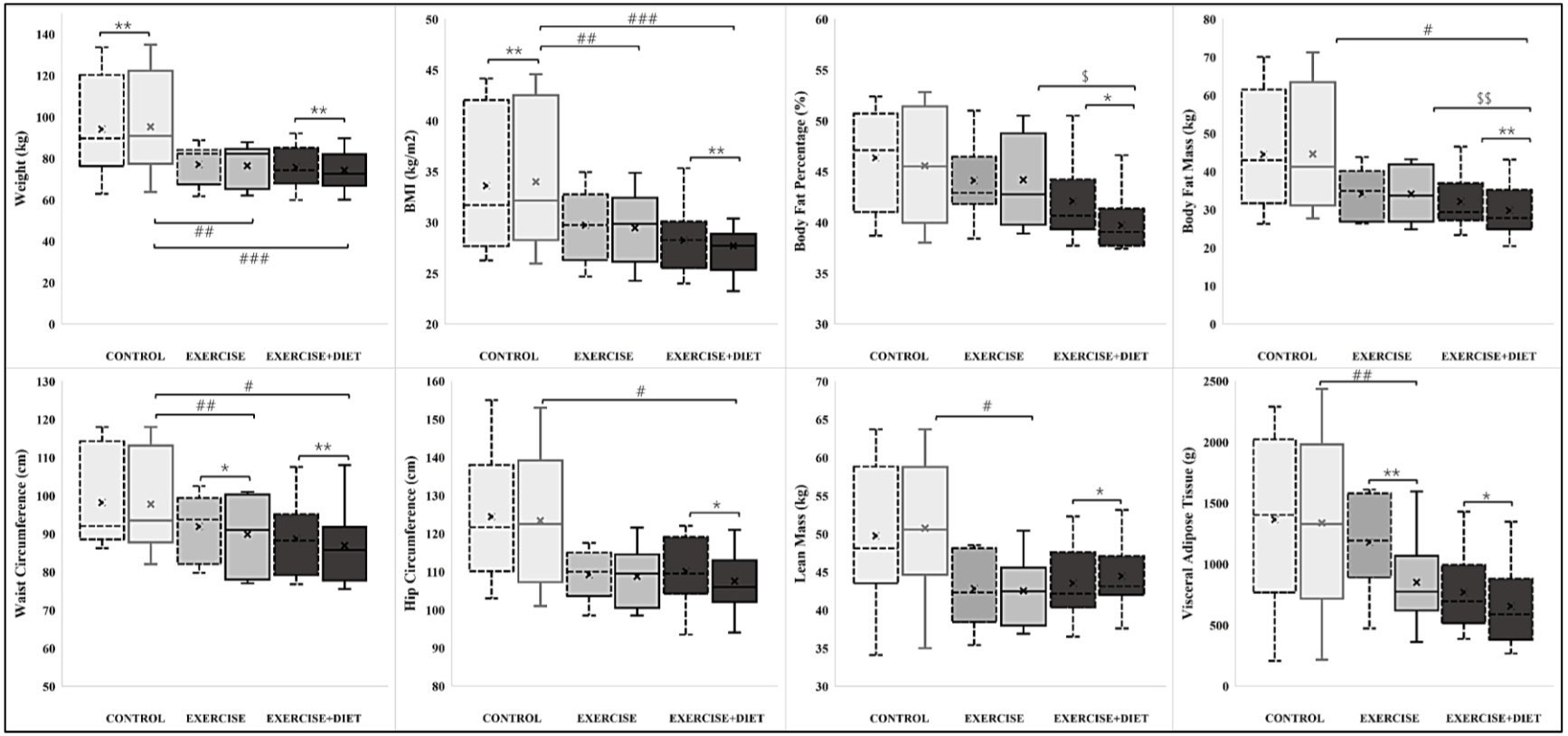

Figure 6.4 Body composition variables of weight, BMI, waist and hip circumference, body fat percentage and mass, lean mass and visceral adipose tissue during baseline and post-intervention in control (CTL), exercise (EX) and exercise+diet (EX+D) groups. Adjusted model controlling for baseline values as covariate for between group effects, and paired t-test or Wilcoxon signed-rank test for within group for normally distributed and not normally distributed respectively. $^{\$}$ denotes $p < 0.05$ between intervention groups (EX and EX+D), $^{\#}$ denotes $p < 0.05$ when compared to CTL, and * denotes $p < 0.05$ for within group effects. $^{\$\$}$, $^{\#\#}$ and ** represents $p < 0.01$ and $^{\#\#\#}$ represents $p < 0.001$. The line within the box plots represents the median, the lower and upper limits of the box are the 25th and 75th percentiles and the error bars are the 10th and 90th percentiles. Error bars in the line graphs are presented as standard error of the mean.

6.4.2.2 Blood Pressure

There were no differences in baseline SBP and DBP ($p > 0.05$).

There were significant intervention effects for DBP between the study groups ($F_{(2, 29)} = 6.51$, $p = 0.005$), but not for SBP ($F_{(2, 29)} = 0.40$, $p = 0.68$). Compared to CTL, DBP significant decreased in EX ($p = 0.028$) and EX+D ($p = 0.001$).

Post-intervention, there were no differences in SBP for all groups ($p > 0.05$).

In EX and EX+D, there were significant decreases in DBP by 4 mmHg ($p = 0.011$) and 6 mmHg ($p < 0.001$) respectively. There was no change in DBP for CTL (0.9 mmHg, $p = 0.78$).

6.4.2.3 Lipid, Glycaemic and Insulin Markers

There were no significant differences in baseline lipid, glycaemic or insulin markers between groups ($p > 0.05$).

There were significant intervention effects for insulin ($F_{(2, 29)} = 7.0$, $p = 0.004$), HOMA2%B ($F_{(2, 29)} = 34$, $p = 0.05$), HOMA2%S ($F_{(2, 29)} = 4.1$, $p = 0.028$) and HOMA2IR ($F_{(2, 29)} = 6.8$, $p = 0.004$) between the study groups. Compared to CTL, insulin and HOMA2IR significantly decreased in EX and EX+D ($p < 0.05$). There were also significant decreases in HOMA2%B and HOMA2%S in EX+D when compared to CTL ($p < 0.05$). There were no significant intervention effects for the other lipid and glycaemic variables ($p > 0.05$) (Table 6.4).

Post-intervention in CTL, there were significant increases in insulin by 3.7 mU/L ($p = 0.018$) and HOMA2IR by 0.5 ($p = 0.016$). There was also a significant decrease in HOMA2%S by -30.8 ($p = 0.009$) in CTL. No significant changes were observed for all insulin variables in EX or EX+D ($p > 0.05$). There were no differences in HbA1c, FBG, HDL, TG, TRG and LDL in all groups ($p > 0.05$).

Table 6.4 Baseline and post-intervention lipid, glycaemia and insulin variables in all groups.

	CTL (n = 10)				EX only (n = 10)				EX+D (n = 10)				*P*
	Pre	**Post**	**Δ**	***P***	**Pre**	**Post**	**Δ**	***P***	**Pre**	**Post**	**Δ**	***P***	***(G)***
Lipid Markers (mmol/L)													
TC	5.8 ± 1.1	6.2 ± 1.1	0.4 ± 1.2	0.32	5.9 ± 1.4	6.3 ± 1.1	0.3 ± 1.2	0.42	6.0 ± 0.9	5.9 (5.4, 7.0)	0.3 ± 0.6	0.17	0.99
TRG	1.4 ± 0.6	1.4 ± 0.7	0.0 ± 0.5	0.99	1.3 ± 0.6	1.4 ± 0.4	0.1 ± 0.4	0.45	1.2 ± 0.5	1.3 ± 0.4	0.0 ± 0.5	0.69	0.84
HDL	1.3 (1.0, 1.4)	1.4 (1.2, 1.6)	0.1 ± 0.5	0.33	1.3 ± 0.3	1.3 ± 0.3	0.1 ± 0.2	0.28	1.5 ± 0.5	1.4 (1.3, 1.9)	0.1 ± 0.3	0.36	0.62
LDL	3.9 ± 0.6	4.1 ± 1.0	0.3 ± 0.8	0.31	4.0 ± 1.2	4.2 ± 0.8	0.2 ± 1.0	0.57	3.9 ± 0.5	4.0 ± 0.8	0.1 ± 0.6	0.49	0.93
Glycaemic and Insulin Markers													
FBG mmol/L	5.5 ± 0.6	5.8 ± 0.6	0.2 ± 0.7	0.35	5.3 ± 0.8	5.5 ± 0.8	0.2 ± 0.8	0.63	5.6 ± 0.8	5.5 ± 0.8	0.2 ± 0.8	0.63	0.95
HbA1c %	5.4 ± 0.4	5.4 ± 0.3	0.0 ± 0.1	1.0	5.6 ± 0.3	5.6 ± 0.3	0.0 ± 0.1	0.52	5.4 ± 0.2	5.4 ± 0.2	0.0 ± 0.2	0.57	0.43
Insulin mU/L	8.6 ± 3.4	12.4 ± 4.5	3.7 ± 4.1	**0.018**	11.4 ± 5.0	9.5 ± 3.0$^{\#}$	-1.9 ± 3.5	0.12	8.9 ± 4.3	7.8 ± 2.8$^{\#\#}$	-1.1 ± 3.8	0.39	**0.004**
HOMA2%B	86.1 ± 31.8	103.4 ± 43.3	17.3 ± 34.1	0.17	107.9 ± 34.8	91.6 ± 21.6	-16.3 ± 22.7	0.06	92.7 ± 39.5	79.9 ± 25.1$^{\#}$	-12.8 ± 39.3	0.34	**0.05**
HOMA2%S	98.0 ± 32.9	67.2 ± 20.9	-30.8 ± 29.6	**0.009**	81.6 ± 35.1	84.8 ± 24.8	3.1 ± 21.7	0.39	109.8 ± 63.3	109.6 ± 47.3$^{\#}$	-0.2 ± 65.9	0.51	**0.028**
HOMA2 IR	1.14 ± 0.43	1.64 ± 0.56	0.50 ± 0.53	**0.016**	1.41 ± 0.67	1.27 ± 0.41$^{\#}$	-0.14 ± 0.47	0.15	1.18 ± 0.57	1.05 ± 0.38$^{\#}$	-0.13 ± 0.50	0.43	**0.004**

Data presented as mean ± SD or median (IQR). TC: total cholesterol; TRG: triglycerides, HDL: high-density lipoprotein; low-density lipoprotein; FBG: fasting blood glucose; HbA1c: glycated haemoglobin. $^{\#}$denotes significance compared to CTL. $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$. G: group effect.

6.4.2.4 Metabolic Status and Risk Scores of Total Cohort

Baseline and post-intervention metabolic status variables of frequency of overweight and obese, estimated aerobic capacity, VAI, QRISK3 scores and frequency of MetS were analysed to assess the effects of the intervention (Table 6.5). There were no significant differences between groups for all variables at baseline or post-intervention. At baseline, there were 30% (n = 3) of participants in the CTL that had MetS, while 40% of participants in both EX and EX+D had MetS. Of those participants at post-intervention, two and three participants respectively in EX and EX+D no longer met the MetS criteria, while an additional participant developed MetS in CTL.

At baseline and post-intervention in CTL, EX and EX+D groups, 40% (n = 4), 50% (n = 5) and 80% (n = 8) of participants were overweight respectively, with the rest being obese. In EX, there was a significant increase in estimated aerobic capacity by 2.9 mL/kg/min (p = 0.028) at post-intervention.

Table 6.5 Metabolic status and risk scores at pre- and post-intervention. Overweight and obese based on body mass index ≥25 kg/m^2 and ≥30 kg/m^2 respectively. Metabolic syndrome diagnosis based on criteria based on International Diabetes Federation (Alberti et al., 2009).

		CTL (n = 10)	EX only (n = 10)	EX+D (n = 10)	*P*
Overweight n (%)	**Pre**	4 (40)	5 (50)	8 (80)	0.19
	Post				
	Δ				
	P				
Obesity n (%)	**Pre**	6 (60)	5 (50)	2 (20)	0.19
	Post				
	Δ				
	P				
Estimated Aerobic Capacity (mL/kg/min)	**Pre**	28.6 ± 4.6	30.1 ± 6.0	30.0 ± 4.8	0.09
	Post	28.5 ± 3.8	33.0 ± 7.0	31.6 ± 4.5	
	Δ	0.0 ± 1.9	2.9 ± 3.1	1.6 ± 2.5	
	P	0.67	**0.028**	0.08	
Visceral Adiposity Index	**Pre**	2.2 ± 0.9	1.9 ± 0.8	1.6 ± 0.7	0.63
	Post	2.0 ± 1.1	1.9 ± 0.7	1.5 ± 0.6	
	Δ	-0.2 ± 0.7	0.0 ± 0.6	-0.1 ± 0.6	
	P	0.44	0.43	0.46	
QRISK3 (%)	**Pre**	3.7 ± 1.3	5.0 ± 1.6	4.7 ± 1.7	0.67
	Post	3.7 ± 1.2	4.9 ± 1.4	4.4 ± 1.4	
	Δ	0.1 ± 0.6	-0.1 ± 0.9	-0.3 ± 0.6	
	P	0.76	0.69	0.15	
Metabolic Syndrome n (%)	**Pre**	3 (30)	4 (40)	4 (40)	0.08
	Post	4 (40)	2 (20)	1 (10)	
	Δ	1 (10)	-2 (-20)	-3 (-30)	
	P	0.43	0.17	0.08	

6.4.3 Intervention Effects on Ghrelin and Adipokine Response

There were no differences in baseline AG (p = 0.9), DAG (p = 0.6), leptin (p = 0.4), adiponectin (p = 0.1) or adiponectin:leptin (ADI:LEP) (p = 0.40) between groups (Table 6.6). There was a significant main effect for group DAG (F = 5.4, p = 0.04). There were significant differences in DAG between CTL vs EX (p = 0.04) and EX vs EX+D (p = 0.02). There were no differences between groups for AG, leptin, adiponectin or ADI:LEP (Figure 6.5).

In CTL, there was a significant decrease in DAG by -46.0 (p = 0.01). In EX, there was a significant increase in AG by 48.1 (p = 0.04). There were no differences in both AG and DAG ghrelin, and adipokine response post-intervention for EX+D.

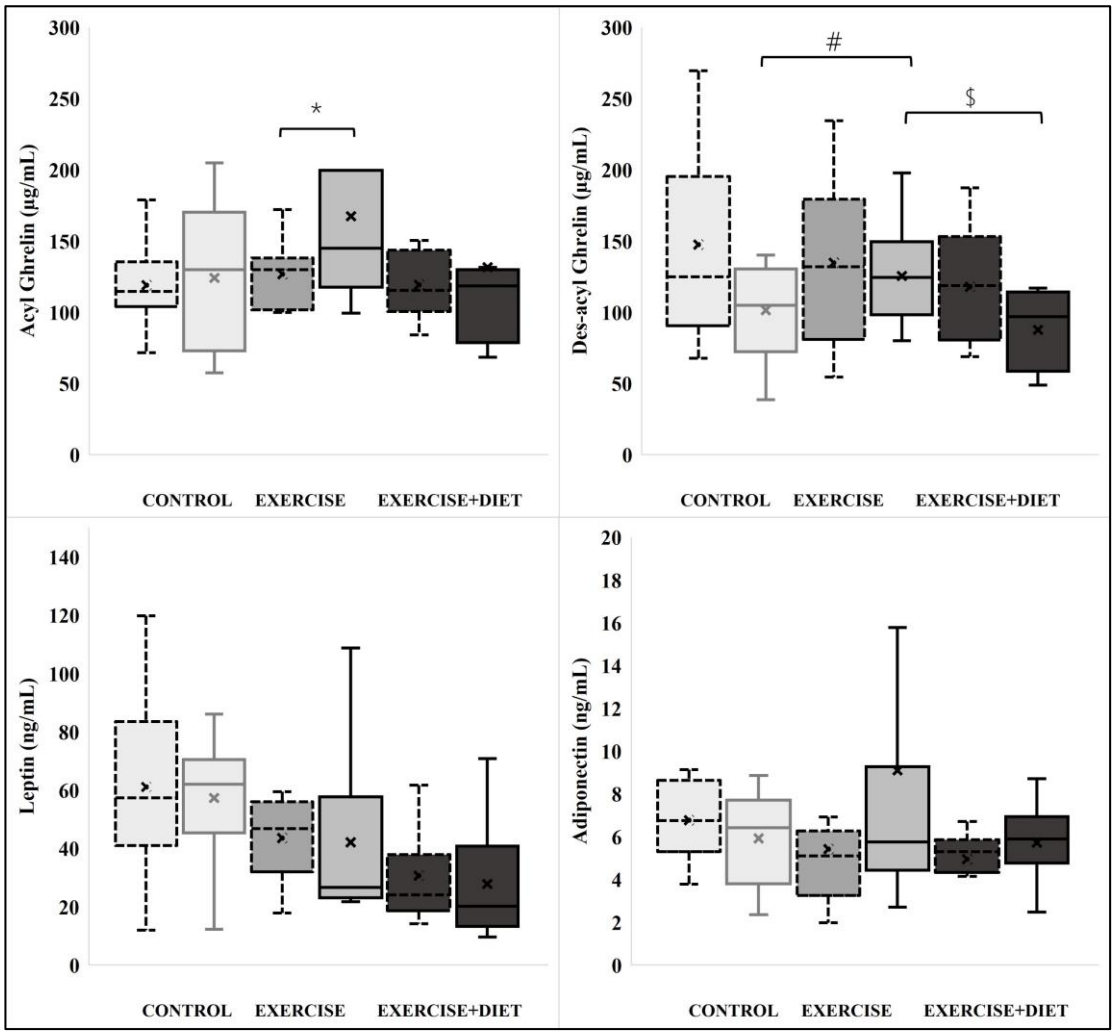

Figure 6.5 Fasting acyl ghrelin, des-acyl ghrelin, leptin and adiponectin levels during baseline and post-intervention in control (CTL), exercise (EX) and exercise+diet (EX+D) groups. Adjusted model controlling for baseline values as covariate for between group effects, and paired t-test or Wilcoxon signed-rank test for within group for normally distributed and not normally distributed respectively. $^{\$}$ denotes $p < 0.05$ between intervention groups (EX and EX+D), $^{\#}$ denotes $p < 0.05$ when compared to CTL , and * denotes $p < 0.05$ for within group effects. The line within the box plots represents the median, the lower and upper limits of the box are the 25th and 75th percentiles and the error bars are the 10th and 90th percentiles. Error bars in the line graphs are presented as standard error of the mean.

Table 6.6 Baseline and post-intervention ghrelin and adipokine in all groups.

	CTL (n = 10)				EX only (n = 10)				EX+D (n = 10)				*P (G)*
	Pre	**Post**	**Δ**	***P***	**Pre**	**Post**	**Δ**	***P***	**Pre**	**Post**	**Δ**	***P***	
Acyl ghrelin* (μg/mL)	125.1 ± 34.6	124.1 ± 51.9	5.1 ± 38.3	0.698	126.7 ± 24.9	144.9 (117.4, 199.5)	12.1 (5.2, 75.5)	**0.046**	119.1 ± 23.9	118.4 (78.6, 129.8)	0.9 (-15.1, 15.2)	0.42	0.52
Des-acyl ghrelin* (μg/mL)	147.4 ± 71.2	101.4 ± 33.2	-46.0 ± 45.8	**0.011**	134.6 ± 58.5	125.4 ± 36.2$^{\#}$	-9.2 ± 19.0	0.64	117.8 ± 40.6	87.6 ± 27.1$^{\$}$	-31.5 ± 67.8	0.069	**0.038**
Leptin (ng/mL)	61.1 ± 31.3	57.3 ± 22.2	9.2 ± 7.2	0.32	43.5 ± 14.1	26.6 (23.0, 57.6)	-1.4 ± 27.4	0.51	24.1 (18.6, 37.8)	20.1 (13.2, 40.7)	-2.8 ± 10.5	0.29	0.44
Adiponectin (ng/mL)	6.8 ± 2.0	5.9 ± 2.3	-0.9 ± 2.9	0.23	5.4 ± 2.9	5.7 (4.4, 10.8)	-0.1 (-0.7, 2.0)	0.27	5.8 (4.7, 21.6)	5.7 ± 1.8	0.7 (-16.0, 1.6)	0.27	0.37
Adiponectin - Leptin Ratio	0.12 (-0.13, 0.01)	0.10 ± 0.04	-0.07 ± 0.11	0.08	0.15 ± 0.10	0.20 (-0.07, 0.41)	0.03 (-0.02, 0.25)	0.11	0.21 ± 0.12	0.28 ± 0.14	0.07 ± 0.12	0.10	0.09

Data are presented as mean ± SD for normally distributed data, and median (IQR) for not normally distributed data. Analysis of covariance employed for main group effect, adjusting for baseline values. *denotes CTL: n = 9, EX: n = 6, EX+D: n = 8 for AG; EX and EX+D n = 9 for DAG. $^{\$}$ denotes $p < 0.05$ between intervention groups (EX and EX+D), $^{\#}$ denotes $p < 0.05$ when compared to CTL, and * denotes $p < 0.05$ for within group effects. G: group effect.

6.4.4 Relationship Between Changes in Ghrelin and Cardiometabolic Risk Markers

Linear regression analysis was employed to establish the relationship between changes in AG and DAG with changes in cardiometabolic risk markers in the total cohort (Table 6.7). No significant associations were found between these markers.

Table 6.7 Linear regression analysis of changes in AG, DAG and markers of cardiometabolic health in total cohort.

	ΔAG			**ΔDAG**		
	Regression Coefficient	**Standard Error**	***P***	**Regression Coefficient**	**Standard Error**	***P***
ΔWeight	-0.007	0.005	0.2	-0.004	0.006	0.57
ΔBMI	-0.003	0.002	0.17	-0.001	0.002	0.53
ΔWaist Circumference	-0.01	0.006	0.1	0	0.007	0.99
Δ%Body Fat	0.003	0.007	0.69	0.01	0.008	0.23
ΔVAT	-0.13	0.87	0.89	-0.89	1.2	0.47
ΔInsulin	-0.005	0.01	0.72	-0.004	0.02	0.83
ΔFasting Blood Glucose	0	0.003	0.97	0.001	0.48	0.69
ΔHbA1c	0	0	0.24	0	0.001	0.81
ΔHOMA2-IR	-0.001	0.002	0.72	-0.001	0.002	0.82
ΔTriglycerides	0.001	0.001	0.28	0.003	0.002	0.07
ΔTotal Cholesterol	-0.003	0.003	0.36	-0.001	0.004	0.77
ΔHDL	-0.001	0.001	0.48	0.001	0.002	0.72
ΔSBP	0.043	0.03	0.15	-0.005	0.03	0.87
ΔDBP	0.005	0.019	0.8	0.009	0.016	0.61

AG: acyl ghrelin; DAG: des-acyl ghrelin; BMI: body mass index; visceral adipose tissue; HbA1c: glycated haemoglobin; HOMA2-IR: homeostatic assessment 2 of insulin resistance; HDL: high-density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure.

6.4.5 Relationship Between Changes in Leptin, Adiponectin and Cardiometabolic Risk Markers

Bivariate correlations were assessed in delta values to establish the relationship between change overtime leptin and adiponectin, and cardiometabolic risk markers in the total cohort. Adiponectin negatively correlated with WC change ($r = -0.38$, $p = 0.04$). Leptin positively correlated with weight ($r = 0.42$, $p = 0.03$) and BMI ($r = 0.43$, $p = 0.02$). ADI:LEP was negatively correlated with weight ($r = -0.54$, $p = 0.003$), BMI ($r = -0.51$, $p = 0.006$) and WC ($r = -0.47$, $p = 0.011$). These associations were mitigated when adjusted for weight.

6.4.6 Adherence to 8-Week Lifestyle Interventions

6.4.6.1 HEFHIIT

Two participants from EX and one participant from EX+D dropped out during the intervention. Health status and family-related reasons were the main factors to participation. No participants were injured during the intervention.

Ten participants each in EX and EX+D completed the intervention. The average adherence rate was 85% (21 out of 24 sessions) and 96% (23 out of 24 sessions) for EX and EX+D respectively. Self-reported HIIT intervals ranged from 20s to 2 mins for the first 4 weeks, and 10s to 60s from weeks 5 – 8. Rest/low intervals ranged from 15s to 4 mins for the first 4 weeks, and 10s to 2 mins during week 5 – 8.

Participants in both groups reached age-related target HIIT zones (≥80% HRmax), with an average HR and average maximum HR of 96.0% and 122.1% respectively for EX, and 98.1% and 123.5% respectively for EX+D. The average rating for RPE scores were 18.4 for EX, and 18.0 for EX+D (Table 6.8).

Table 6.8 Summary of adherence variables to the exercise intervention in both EX and EX+D groups. RPE: rate of perceived exertion; HR: heart rate.

	EX (n = 10)	**EX+D (n = 10)**	***P***
Completed Sessions n (%)	21 (85%)	23 (96%)	0.05
Average RPE	18.4 ± 0.4	18.0 ± 0.8	0.10
Average age-related HR (%)	96.0 ± 6.6	98.1 ± 8.1	0.59
Average age-related maximum HR (%)	122.1 ± 7.2	123.5 ± 6.5	0.69
Average calories expended/session	123.1 ± 34	124.9 ± 22.2	0.50

6.4.6.2 Mediterranean-style Diet

EX+D:

Throughout weeks 0 – 8, there were significant differences between the reported MEDAS scores and MFP logs ($p < 0.05$) except for week 1 ($p = 0.05$) and week 5 ($p = 0.07$) (Table 6.9).

Reported MEDAS: Mean adherence percentages across the eight weeks showed 80% (n = 8) of participants had optimal adherence (Table 6.10). Of the ten participants in the EX+D group, the baseline mean MEDAS scores and percentage were 6.6 ± 2.8 and 56.4 ± 16.9 %, respectively. At post-intervention, all participants improved their adherence to MedDiet, with significant increases in the mean MEDAS score by 4 points (post: 10.6 ± 2.9) and percentage adherence of

23.2% (post: 79.6 ± 17.3; p = 0.002). Throughout the eight weeks, 50% of participants (n = 5) maintained optimal adherence to the MedDiet (92.6 ± 0.1 %). Of the remaining participants, 30% (n = 3) maintained optimal adherence (77.8 ± 0.1 %) for seven weeks, 10% (n = 1) for two weeks (71.4 ± 0.0), and the remaining participant did not adhere throughout the eight weeks (56.8 ± 0.1 %).

MyFitnessPal logs: According to the FMP logs, mean adherence percentages across the eight weeks showed 60% (n = 6) of participants had optimal adherence (Table 6.10) The mean baseline MEDAS score and percentage were 4.6 ± 2.0 and 34.3 ± 13.9 %, respectively. At post-intervention, all participants improved their adherence to the MedDiet, with significant increases in the mean MEDAS score by 4.5 points (post: 4.6 ± 2.0) and percentage adherence of 34.2% (post: 9.1 ± 2.6; p < 0.001). Throughout the eight weeks, 40% of participants (n = 4) maintained optimal adherence to the MedDiet (83.6 ± 0.1 %). Of the remaining participants, 10% (n = 1) maintained optimal adherence for 6 weeks (72.6 ± 0.0 %), 30% (n = 3) for 1 - 4 weeks (73.3 ± 0.0), and 20% (n = 2) did not adhere throughout the eight weeks (47.6 ± 0.1 %).

Table 6.9 Comparison of MEDAS scores between self-reported MEDAS vs MyFitnessPal logs.

MyFitnesspal / MEDAS	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Week 0	**0.003***								
Week 1		0.052							
Week 2			**0.018***						
Week 3				**0.012***					
Week 4					**0.006****				
Week 5						0.065			
Week 6							**0.007****		
Week 7								**0.017***	
Week 8									**0.022***

CTL and EX:

To assess the effects of the MedDiet in EX+D group, adherence to MedDiet from MFP dietary logs were also assessed in the CTL and EX groups (Table 6.10). The scores for EX+D significantly differed to CTL and EX groups (p < 0.001) across all parameters of data collected for MedDiet: MEDAS score (F = 18.2, p < 0.001), MEDAS score percentage (F = 22.0, p < 0.001) and average adherence (F = 78.6, p < 0.001).

Both groups did not reach optimal adherence across all weeks, and average adherence were 30.2% and 19.0% for CTL and EX groups respectively. There were no differences between baseline and post-intervention MEDAS percentages for both groups.

Table 6.10 Mediterranean diet adherence from MyFitnessPal logs and reported MEDAS for EX+D group at baseline and follow-up visits.

	MEDAS from MyFitnessPal Logs									***P*** **(G)**	Reported MEDAS (EX+D)		
	CTL			**EX**			**EX+D**						
	PRE	**POST**	***p***	**PRE**	**POST**	***p***	**PRE**	**POST**	***p***		**PRE**	**POST**	***p***
MEDAS score*	4.2 ± 2.1	3.7 ± 1.8$^{###}$	0.60	3.1 ± 1.6	3.0 ± 1.1$^{###}$	0.76	4.6 ± 2.0	9.1 ± 2.6	**0.003**	**<0.001**	6.6 ± 2.8	10.6 ± 2.9	**0.003**
MEDAS score %*	29.8 ± 15.3	26.2 ± 7.3$^{###}$	0.60	24.0 ± 13.0	22.6 ± 8.0$^{###}$	0.66	34.3 ± 13.9	68.5 ± 16.2	**0.002**	**<0.001**	49.2 ± 18.1	79.6 ± 17.3	**0.002**
Improved n$		2			3			10		0.20		10	
Across 8 weeks													***P (G)***
Average Adherence %*	30.2 ± 8.8$^{###}$			19.0 ± 6.8$^{###}$			68.2 ± 16.3			**<0.001**	80.9 ± 14.5		**< 0.001**
Maintained average optimal adherence n (%)$	0 (0%)			0 (0%)			6 (60%)			0.23	8 (80%)		0.23
8 weeks adherence n (%)$	0 (0%)			0 (0%)			4 (40%)			0.22	5 (50%)		0.22
5 – 7 weeks adherence n (%)$	0 (0%)			0 (0%)			1 (10%)			0.22	3 (30%)		0.22
1 – 4 weeks adherence n (%)$	0 (0%)			0 (0%)			3 (30%)			0.22	1 (10%)		0.22
0 weeks adherence n (%)$	0 (0%)			0 (0%)			2 (20%)			0.22	1 (10%)		0.22

Data are presented as mean ± SD for normally distributed data. *Analysis of covariance employed for main group effect, adjusting for baseline values. $ Categorical data tested using Pearson Chi-squared test. $^{###}$ denotes $p < 0.001$ and significance compared to EX+D. G: group effect.

6.4.7 Nutrient Values, Caloric Expenditure and Habitual Physical Activity

In all groups, nutrient values, caloric expenditure and step count did not change across 8 weeks (Table 6.11).

Table 6.11 Nutrient values from MyFitnessPal in all groups.

		CTL (n = 10)	EX only (n = 10)	EX+D (n = 10)	*P*
Total Energy (kcal/day)	**Pre**	1408.4 ± 217.4	1230 (1150.2, 1402.4)	1527.3 ± 348.1	0.2
	Post	1251.4 ± 132.6	1402.3 ± 351.7	1391.3 ± 236.1	
	Δ	-157.0 ± 182.2	88.7 ± 163.1	-136.1 ± 300.4	
	P	0.09	0.12	0.19	
Carbohydrate (g)	**Pre**	148.0 ± 34.0	144.8 ± 29.0	156.1 (116.5, 173.3)	0.22
	Post	123.7 ± 10.9	129.7 ± 24.5	152.8 ± 46.2	
	Δ	-24.2 ± 35.8	-15.1 ± 30.5	-5.3 ± 25.4	
	P	0.16	0.21	0.52	
Fat (g)	**Pre**	53.5 ± 13.0	46.1 ± 22.0	62.9 ± 25.7	0.9
	Post	49.2 ± 7.6	51.8 ± 18.3	53.6 ± 20.0	
	Δ	-4.3 ± 12.1	5.7 ± 14.2	-9.3 ± 33.1	
	P	0.43	0.29	0.39	
Protein (g)	**Pre**	57.2 ± 15.0	51.8 ± 13.8	56.9 ± 15.0	0.25
	Post	50.4 ± 14.2	58.1 ± 17.4	56.0 ± 16.1	
	Δ	-6.7 ± 9.2	6.3 ± 12.6	-0.9 ± 4.4	
	P	0.13	0.2	0.85	
Steps	**Pre**	6101 ± 3540	5946 ± 2523	6485 ± 1939	0.18
	Post	5292 ± 1974	7316 ± 3621	7548 ± 2824	
	Δ	-313	1370 ± 4350	1063 ± 2406	
	P	0.48	0.35	0.2	
Active Expenditure (kcal)	**Pre**	528.7 ± 342.3	429.2 ± 264.3	437.2 ± 171.8	0.84
	Post	504.3 ± 277.1	468.2 ± 273.6	415.5 ± 219.2	
	Δ	-19.5 (2.4, 66.6)	47.4 ± 313.4	-21.7 ± 169.3	
	P	0.86	0.66	0.70	
Rest Expenditure (kcal)	**Pre**	1824.8 ± 314.7	1591.3 ± 142.6	1595.9 ± 162.9	0.90
	Post	1797.1 ± 341.8	1557.4 ± 129.8	1580.6 ± 159.1	
	Δ	0 (-31.2, 17.0)	0 (-5.7, 24.9)	-15.3 ± 25.5	
	P	0.47	0.47	0.09	
Total Expenditure (kcal)	**Pre**	2353.5 ± 527.4	2020.4 ± 281.9	2033.1 ± 124.9	0.74
	Post	2301.5 ± 572.8	1920.1(1833.0, 2288.0)	1996.1 ± 190.1	
	Δ	27.2 (-6.0, 45.3)	15.1 ± 278.4	-37.0 ± 182.2	
	P	0.86	0.86	0.54	

Data are presented as mean ± SD for normally distributed data, and median (IQR) for not normally distributed data. Analysis of covariance employed for main group effect, adjusting for baseline values. *denotes $p < 0.05$; $^{\#}$ denotes significance compared to control group; $^{\$}$ denotes significance between intervention groups (EX vs EX+D).

6.5 Discussion

The main objectives of this study were to investigate the cardiometabolic and ghrelin response to HEFHIIT with/without MedDiet, as well as to explore their relationship in inactive, overweight post-menopausal women. In accord with the first hypothesis of this study, there were an enhanced body composition profile in EX+D compared to EX, with significant improvements in VAT and DBP observed in both intervention groups. Additionally, CRF also significantly improved in the HEFHIIT group. Notably, the lack of lifestyle intervention (CTL group) resulted in significant increases in body weight and IR. AG and DAG differed between the intervention groups at the end of 8 weeks, corresponding with the second hypothesis. Specifically, EX+D exhibited unchanged AG and DAG, while EX demonstrated significant increase in AG with unchanged DAG. In disagreement with the third hypothesis, this study failed to establish a relationship between the changes in the ghrelin isoforms and cardiometabolic risk markers.

6.5.1 Cardiometabolic Response to the Interventions

It is established that post-menopausal women have higher susceptibility to gains in VAT due to shifts in body composition, resulting in an increased cardiometabolic risk (Kodoth et al., 2022). The supplementation of the MedDiet to HEFHIIT elicited favourable weight loss and body compositional profile compared to HEFHIIT alone. In agreement, meta-analyses investigating the integration of diet and exercise have consistently demonstrated more favourable cardiometabolic health outcomes compared to diet or exercise in overweight post-menopausal women (Cheng et al., 2018; Khalafi & Symonds, 2023). Notably, both Cheng *et al.* (Cheng et al., 2018) and Khalafi *et al.* (Khalafi & Symonds, 2023) independently reported superior improvements in body composition with combined exercise and diet compared to standalone interventions. Furthermore, the UK Women's Cohort Study found adherence to MedDiet over 4-years to be associated with smaller increases in WC and reduced risk of abdominal obesity in post-menopausal women (Best & Flannery, 2023). In both intervention groups, significant VAT reductions were observed that were absent in CTL. Findings support high-intensity interval exercise (HIIE) in improving post-exercise energy homeostasis and metabolic adaptations, potentially due to its ability to increase post-exercise fat oxidation and thereby mitigating body fat deposition (Atakan et al., 2022). In accordance with previous meta-analyses, performing HIIT for at least 4 weeks have demonstrated efficacy in reducing abdominal adiposity (Andreato et al., 2019; Guo et al., 2023), supporting our findings. This study shows that HEFHIIT with or without the MedDiet can improve abdominal obesity in predisposed women, an important factor in mitigating cardiometabolic dysfunction.

In addition to abdominal adiposity, significant reductions in DBP were observed in both intervention groups, with no differences between groups. Similar to the findings of Chapter 5, there is a wide consensus from previous meta-analyses on the benefits of HIIT in improving BP in healthy individuals (Batacan et al., 2017), older adults (Carpes et al., 2022), coronary artery disease (Chen & Tang, 2020) and hypertension (de Souza et al., 2022). Thus, confirming that HEFHIIT can decrease DBP. Additionally, the 4-years observational PREDIMED study found no

changes in SBP but found decreases in DBP with the MedDiet and exercise group (Toledo et al., 2013), corroborating the findings of this study. Here, HEFHIIT resulted in reductions of -4 mmHg in DBP, while the combination of HEFHIIT and MedDiet led to a reduction of -6 mmHg in DBP, although these changes were not significant of each other. In disagreement, a systematic review has reported that the combination of exercise and dietary interventions have increased DBP lowering effectivity (Jurik & Stastny, 2019). However, it should be noted that these studies utilised strength training and were conducted in pre-hypertensive and hypertensive individuals, potentially explaining the discrepancy between our findings. While the MedDiet offers cardiometabolic benefits, the question raised was the potential of the specific food component, olive oil, as the factor for eliciting favourable effects on DBP. Dietary analysis in this study found the increased consumption of olive oil to be the greatest change in MedDiet components. Previous studies have demonstrated the vasoprotective effect of polyphenols present in olive oil on BP by increasing endothelial synthesis of nitric oxide (NO) (Sarapis et al., 2020). Thus, it is uncertain if improvements in DBP were attributed to the MedDiet and/or the increased consumption of olive oil. Further research is required to establish the individualistic effects of the MedDiet as well as olive oil supplementation alone to distinguish improvements on cardiometabolic health with the MedDiet. Nevertheless, the participants in this study were not hypertensive, highlighting the effectiveness in reducing DBP with HEFHIIT with or without the MedDiet in non-hypertensive post-menopausal women.

6.5.2 Declines in Cardiometabolic Health in Control Group

While investigating into the effects of continuous physical inactivity and habitual diet were not an objective in this study, indicators of cardiometabolic declines were evident through significant weight gain, increase in insulin levels and IR, as well as attenuated insulin sensitivity in the CTL group over the 8 weeks that warrants further discussion. The relationship between physical inactivity and IR is established (Yaribeygi et al., 2021). Trajectories involving weight gain contribute to the dysregulation of glucose metabolism and decreased insulin sensitivity (Walsh et al., 2018). While the exact pathophysiology is not fully understood, one proposed mechanism involves islet cell insufficiency. An inactive lifestyle can increase islets' workload and decrease their efficiency, leading to IR. In contrast, regular physical activity and engagement in exercise can preserve and restore islet function, thus enhancing insulin sensitivity (Yaribeygi et al., 2021). Consistent with our findings, eight months of inactivity in overweight middle-aged adults resulted in significant increase in FBG and IR, which was ameliorated with moderate to high-intensity exercise (Slentz et al., 2009). Furthermore, the Western diet characterised by high intakes in saturated fat, processed food and sugar, is recognised for its association with hyperinsulinemia and the pathogenesis of MetS, among other detrimental effects on cardiometabolic health (Clemente-Suárez et al., 2023). As expected, the MedDiet adherence in CTL group was less than optimal (30.2%). Although MedDiet adherence scores were even lower in EX (19.0%), the absence of declines in insulin sensitivity is likely due to HEFHIIT, highlighting that even without

the MedDiet, regular physical activity is paramount in mitigating cardiometabolic risk progression in overweight/obese post-menopausal women.

6.5.3 AG and DAG Response to the Intervention

Opposing AG and DAG response following 8 weeks was observed between both intervention groups in this study. The response of ghrelin to chronic exposure of exercise interventions have been extensively studied. The systematic review by Ouerghi *et al.* observed a consensus of significant increase in TG and DAG following long-term (≥12 weeks) and very long-term (≥12 months) exposure to exercise, particularly evident with weight loss in overweight/obese individuals (Ouerghi et al., 2021). In disagreement, significant increases in AG were observed with the absence of weight changes following 8 weeks of HEFHIIT of the present study. Alterations in circulating TG is an important adaptation to weight loss, serving as a compensatory signal for body weight restoration (Briggs et al., 2013). A key strength of this study is the evaluation of both ghrelin isoforms. Given the limited studies on the response of AG to chronic exercise, it can only be theorised that the observed increase in AG acts as an initial protective mechanism against elevated EE, thereby preventing weight gain to safeguard the body weight set point (Churm et al., 2017). The repeated bouts of HEFHIIT may trigger counterregulatory systems by augmenting AG, thus increasing appetite to match the increase in exerted EE, as well as to protect the initial set weight point. Despite greater suppression of AG in an intensity-dose manner that translates to greater appetite suppression following acute HIIT (Anderson et al., 2021), this does not directly translate into decreased caloric intake, as evidenced by unchanged total daily EI following chronic exposure to HIIT (≥4 weeks) (Taylor et al., 2018). Consistent with previously mentioned studies, the significant increase in AG in this was accompanied by unchanged EI and weight in EX. Furthermore, significant reductions in VAT independent of weight loss may be attributed by the high fat oxidation post exercise capacity of HIIT (Atakan et al., 2022).

Despite significant weight loss observed in the EX+D group, no changes in AG or DAG were found in this study. There is limited body of research investigating the combined effects of MedDiet and exercise on ghrelin levels, with only two studies conducted thus far (Hernando-Redondo et al., 2022; Tsaban et al., 2022). This scarcity poses a challenge in understanding the underlying mechanisms of ghrelin alterations in this specific cohort. The PREDIMED-PLUS study involving a mixed cohort of middle-aged adults found an initial reduction (6 months) followed by a slight increase in circulating TG at the end of the 12-months intervention, with no statistical significance (Hernando-Redondo et al., 2022). In another study with similar cohort, Tsaban *et al.* reported significant increase in TG with an 18-month caloric restricted MedDiet with physical activity (Tsaban et al., 2022). Collectively, these studies suggests that long-term exercise (≥12 weeks) with or without the MedDiet lead to an increase in TG, likely driven by DAG increase, contradicting the outcomes of this study evaluating an 8-week intervention.

Although it is unclear, the variations in AG response to the intervention could be attributed to the addition of the MedDiet. In this study, the greatest change in MedDiet components were the

increased consumption of olive oil and nuts. Olive oil, rich in an oleic acid, is a monounsaturated fat with long-chain fatty acids, and is proposed to be a potential inhibitor of ghrelin octanoylation. Oiso *et al.* found oleic acid to have the strongest suppressive effect on AG production (Oiso et al., 2015), mirroring findings in this study. This is also supported by another study that found greater decrease in AG following an isolated dose of extra virgin olive oil in individuals with morbid obesity (Garcia-Serrano et al., 2021). Although the type of olive oil was not specified in diary reporting of this study, it should be noted that all olive oil types contain at least 55% oleic acid. The consumption of oleic acid has been linked to the suppression of ghrelin secretion from the stomach cells (Oiso et al., 2015) that correspond to the suppression in subjective appetite and increased satiety (Prater et al., 2023). High adherence to MedDiets have been associated with 2-fold increase in the likelihood of weight loss maintenance (Poulimeneas et al., 2020). The observed suppression of AG production by oleic acid in MedDiets may contribute to mitigating body weight gain. Ghrelin is a potent regulator of energy metabolism, with ghrelin levels increasing to compensate for increased EE, hence promoting fat storage to protect the body weight set point (Briggs et al., 2013). We theorise that the unchanged AG levels potentially due to increased consumption of oleic acid may counteract lipid and adiposity accumulation as well as mitigate compensatory increase in EI, thereby fostering favourable body composition changes in an isocaloric environment. In line with this, this further supports the theory that the significant increase in TG observed in previous studies is driven by DAG. However, the short duration of 8-weeks poses as a limitation in this study to fully assess the effect of the intervention on ghrelin response.

6.5.4 Relationship Between Lifestyle Intervention-Induced Changes in Ghrelin and Cardiometabolic Risk Markers

Previous studies have reported on the increases in ghrelin levels and improved insulin sensitivity resulting from weight loss (Barazzoni et al., 2021; Mason et al., 2015), supporting the notion for the association between lifestyle intervention-induced ghrelin increases and improvements in cardiometabolic health. In this study, we failed to establish a relationship between the changes in ghrelin and cardiometabolic risk markers associated with the interventions. Although weight loss (-1.5 kg) and VAT reductions were observed in the EX+D group, it is theorised that this was insufficient to establish relationships indicative of ghrelin-mediated improvements in cardiometabolic health. Adiposity, particularly VAT, is associated with glucose homeostasis and insulin secretion (Wondmkun, 2020). It has been noted that the achievement of a minimum 5% reduction in total body weight is associated with diminished metabolic disturbances and serves as a strong predictor of improved IR in individuals with obesity (Magkos et al., 2016). In this study, the significant reductions in weight loss in EX+D equated to only a 2% reduction in total body weight. As previously noted, combined diets and exercise frequently integrate caloric restriction, potentially accounting for the lack of notable changes in cardiometabolic blood markers within the limited 8-week timeframe of this study. It is proposed that extended exposure and/or sustained implementation of caloric restriction aiming for a minimum of 5% reduction in total body weight,

will yield greater reductions in VAT, IR and other aspects of cardiometabolic health and thus allowing a better understanding in ghrelin-mediated improvements in cardiometabolic health.

6.5.5 Relationship between Lifestyle Intervention-Induced Changes in Leptin, Adiponectin and Cardiometabolic Risk Markers

No changes in leptin or adiponectin were observed across all groups. Although existing literature links alterations in leptin and adiponectin with exercise and/or diet-induced weight loss, this association was not evident in our study despite significant weight loss observed in the EX+D group. The discrepancy may stem from the insufficient weight loss to elicit substantial changes in leptin and adiponectin. A meta-analysis by Khalafi *et al.* found a decrease of 6 kg body weight that was associated with leptin and adiponectin change with combined exercise and diet (Khalafi et al., 2023). In a similar cohort to this study, a 3-month weight loss program (hypocaloric diet and exercise) (Wooten et al., 2022) and 6-month hypocaloric diet (Van Rossum et al., 2000) resulted in significant weight loss that also strongly associated with leptin and adiponectin change. This suggests that weight loss irrespective of method employed, is the primary determinant for changes in these adipokines. Notably, correlation analysis in this study revealed positive associations with leptin, weight and BMI change, as well as negative associations between adiponectin and WC change in the total cohort. The ADI:LEP also showed negative correlations with weight, BMI and WC changes. These findings align with the physiological roles of leptin in controlling EE and body weight through the hypothalamus (Friedman & Halaas, 1998), and adiponectin in supporting healthy adipose tissue expansion (Nguyen, 2020). Interestingly, no correlations were found between adiponectin and VAT. In disagreement with the study hypothesis, no correlations were established between leptin and adiponectin changes with energy balance variables. This suggests that weight loss and WC, independent of energy balance, is the primary driver for changes associated with leptin and adiponectin in overweight and obese post-menopausal women respectively. Further analysis is warranted to explore the role of both leptin and adiponectin on other cardiometabolic risk markers.

6.5.6 Adherence

The overall compliance and adherence to the exercise intervention were excellent in both intervention groups, with at least 85% of sessions completed. Furthermore, participants in both groups surpassed the prescribed HEFHIIT of at least 80% HRmax for both average and maximum age-related %HRmax.

Based on the self-reported MEDAS scores, adherence to the MedDiet in EX+D improved significantly from baseline of 49.2% to 79.6% at week 8. To ensure validity of the MEDAS scores, daily logs from self-reported MFP logs were cross-referenced with MEDAS. Despite significant improvements in MedDiet adherence from MFP, this was consistently lower across all weeks compared to the self-reported MEDAS scores. Other studies who investigate adherence to MedDiet interventions typically rely solely on MEDAS (Babio et al., 2014; Garcia et al., 2016), potentially leading to an overestimation of adherence and compliance to the evaluated dietary

intervention. Given the inherent difficulty in accurately documenting individual diets, particularly when relying on self-reported instruments like MFP used in this study, there is a potential for dietary misreporting (Ravelli & Schoeller, 2020). Therefore, results of the present study used MEDAS scores from MFP logs to minimise these errors.

While the results in EX+D suggest overall moderate compliance with the MedDiet, a number of elements from the 14-item questionnaire showed poor compliance. Particularly, a very low proportion of participants achieved high adherence scores for legumes, fish, sweets/pastries and olive oil consumption. The reported low intake of fish corresponds to the average UK intake, while the low adherence to the olive oil recommendations suggests a reflection of generational ideology of 'fear of fat'. National dietary guidelines aimed to reduce coronary heart disease by reducing fat intake were introduced between 1977 to 1983 by the UK government, a time that coincided with the participants in this study. Despite the health benefits of olive oil consumption in scientific literature, the discrepancy between scientific knowledge and consumer beliefs can deter dietary fat consumption altogether, contrasting the MedDiet recommendations.

6.5.7 Strengths and Limitations

One of the strengths of this study was the adherence and compliancy to the prescribed interventions in both groups, with <20% drop-out rate in either group. Poor adherence is known to be one of the pitfalls of exercise and dietary interventions that can hinder favourable outcomes. Lack of time and competing priorities are the main barriers to adherence to lifestyle behavioural modification. Additionally, the utilisation of the DEXA to precisely quantify VAT mass also serves as another strength. While WC serves as a surrogate marker for VAT, it has limitations in accurately representing VAT. Excess abdominal adiposity is the forefront of CMD pathophysiology. Not only do findings from this study highlight the feasibility of HEFHIIT and MedDiet in an unsupervised manner, but also highlight the benefit of improving abdominal obesity and hypertension in a short duration of 8 weeks.

This study is not without limitations. Firstly, a larger sample size would have allowed a better insight into the response to both exercise and diet, given the interindividual variation in compensatory responses with lifestyle interventions such as exercise (Mansfeldt & Magkos, 2023). Secondly, AG is underpowered in EX with n = 6, thus the effects of exercise-mediated alterations in AG in EX should be noted with caution. Furthermore, the incompatibility of the kits disallowed the analysis of AG:DAG ratio and TG, which may have allowed better insight into the relationship between both isoforms. The short 8-week duration have been insufficient in eliciting better cardiometabolic benefits. Additionally, the CTL group exhibited significantly higher BMI than the intervention groups, potentially affecting metabolic health mediation. Nevertheless, the conclusion of the study should be minimally affected due to insignificant differences in EI within groups at baseline and post-intervention, Furthermore, metabolic parameters did not differ between groups at baseline.

Standardisation of olive oil type (particularly with extra virgin olive oil (Garcia-Serrano et al., 2021) should be incorporated in future studies. Furthermore, investigation into the supplementation of oleic acid only may aid in delineating its consumption and effects on ghrelin levels. Finally, the estimation of EI was based on self-reported logs on MyFitnessPal by the participant. Underreporting nutrition on food diaries are common and may have impacted the accuracy of EI estimation. Furthermore, EE was estimated via accelerometer watches worn by the participant which may be inaccurate (Evenson & Spade, 2020). Thus, the isocaloric nature of the participants in this study should be interpreted with caution.

6.6 Conclusion

This study demonstrated excellent adherence to home-based, equipment-free, HIIT (HEFHIIT) with or without the Mediterranean-style diet (MedDiet). The combination of both behaviours show superiority to HEFHIIT alone in benefiting weight loss and body composition. In line with the EMAS position statement, this study reiterates the benefits of MedDiet adherence as well as the incorporation of HEFHIIT in improving abdominal adiposity in post-menopausal women. The reductions in abdominal adiposity associated with this lifestyle behaviour translates into favourable cardiometabolic profile if maintained for a prolonged period. Notably, indications of cardiometabolic health decline through significant increases in weight and insulin resistance were observed in the control group.

Acyl ghrelin mediation was observed with HEFHIIT, independent of weight and energy balance changes. In expansion, the addition of the MedDiet resulted in significant body compositional improvements, independent of ghrelin mediation and energy balance changes. The varying adaptive response of ghrelin to HEFHIT with or without MedDiet suggests that mediation of ghrelin is independent of weight changes. While no relationship was established between ghrelin mediation and cardiometabolic risk markers in the intervention groups, the relationship between changes in AG and DAG with weight and VAT in the control group further supports the role of ghrelin in the pathophysiology of metabolic-related disorders.

Chapter 7 – General Discussion

7.1 General Discussion

The understanding of the influence on cardiometabolic risk markers with exercise and diet and the mediatory role of the ghrelin axis in predisposed post-menopausal women is limited. Therefore, the aim of this PhD was to develop and implement an easily accessible lifestyle intervention programme that could mitigate cardiometabolic risk while exploring the role of ghrelin in physically inactive, overweight post-menopausal women.

This project details the cardiometabolic benefits of incorporating exercise training of at least 8 weeks in post-menopausal women. Furthermore, home-based activity such as HEFHIIT with or without maintaining caloric intake for 8 weeks can improve at least one cardiometabolic benefit. When maintaining caloric intake, HEFHIIT with the MedDiet achieved greater weight loss alongside better body composition with combined interventions, with significant improvements in VAT and DBP with both interventions of HEFHIIT with/without MedDiet. Notably, the absence of positive lifestyle behaviours (i.e. diet and exercise) resulted in significant increases in body weight and a reduction in insulin sensitivity over the same 8 weeks. The findings from this thesis did not establish any relationships between changes in ghrelin isoforms and cardiometabolic risk markers.

7.2 Adherence and Compliance to the Lifestyle Interventions

It is important to address the adherence and sustainability of the interventions in this study before diving into the outcomes of interest. Public health guidelines recommend regular physical activity of moderate intensity of at least 150 minutes, or 75 minutes of vigorous physical activity a week to maintain health or prevent major chronic diseases (Davies et al., 2019). However, many women in this population do not achieve this and perceived barriers and facilitators to lifestyle behavioural modifications often impede participation, preventing one from leading a healthier lifestyle (Spiteri et al., 2019). The most common barriers predominate around time constraints and competing priorities, with the emphasis for the need for the diet and/or exercise to seamlessly integrate into routine for effective behaviour change (Deslippe et al., 2023).

The home-based nature likely contributed to the high adherence and compliance throughout the 8 weeks. While poor adherence is evident in unsupervised exercise interventions (Bannell et al., 2023), it has been cited that factors that contribute include lack of knowledge, personalisation and support from the exercise specialists (Morgan et al., 2016). It is important to note that while the nature of the study intervention was home-based and unsupervised, support through continuous contact was ensured throughout the 8 weeks. Furthermore, a two-way comprehensive briefing at pre-intervention enabled the researcher's understanding of each participant's needs and ability as well as the participant's understanding of the intervention to maximise support remotely. In the exercise groups, all participants were equipped with a smart watch (accelerometer) that allowed individual monitoring of feedback during exercise (exercise intensity and heart rate) and the flexibility of utilising fitness videos (YouTube) allowed tailoring to individual preferences. In

combination with remote support (i.e. text messages from the researcher) for both intervention groups, these elements facilitated excellent adherence and compliance. While the small cohort may limit generalisability, the adherence aligns with a larger study that investigated home-based, unsupervised exercise utilising a combination of the previously mentioned elements (Bannell et al., 2023). In a study sample of 86 participants, Bannell *et al.* found positive impact on adherence, allowing participants to meet recommended exercise guidelines (Bannell et al., 2023).

However, it is crucial to discuss the relatively poor adherence to the prescribed exercise intensity of ≥80% HRmax, despite good attendance adherence. The use of accelerometers allowed us to monitor exercise intensity, and data indicated that participants frequently engaged in moderate-intensity training rather than the prescribed HIIT. This discrepancy may be attributed to several factors, including individual discomfort with high-intensity exertion, misinterpretation of intensity guidelines, or a preference for moderate-intensity exercises that felt more sustainable and less physically taxing.

Moderate-intensity training, while beneficial, may not provide the same cardiometabolic benefits as high-intensity training in shorter sessions. Studies show that moderate-intensity continuous training (MICT), performed thrice weekly for 60 minutes, yields better outcomes compared to HIIT sessions lasting 15 – 40 minutes (Yin et al., 2023). These findings suggest that exercise duration is crucial for intensity-related benefits. Future interventions should implement strategies to ensure participants reach targeted intensity levels, such as detailed education on intensity perception, immediate feedback during exercise, or periodic supervised sessions to reinforce high-intensity practices.

Collectively, this thesis demonstrates the feasibility of home-based interventions and highlights the importance of support and flexibility to foster optimal adherence to exercise and dietary interventions. It also underscores the need for careful monitoring and strategies to ensure adherence to prescribed exercise intensities, as this is critical for achieving the desired health outcomes. It should be noted that adherence was not the primary focus of this study, and the relatively short 8-week duration may have supported higher adherence, potentially not reflective of longer-term interventions. Nevertheless, the high adherence and compliance observed in this study contribute to the validity of the outcomes of interest.

7.3 Summary of Cardiometabolic Health Markers

This section collates the cardiometabolic health outcomes from the experimental chapters (Chapters 5 and 6) and place these observations into context within the meta-analysis in Chapter 4 and existing literature.

MetS is the precursor of CMD progression. In Chapter 4, the main findings of this meta-analysis extend public health guidelines from organisations including the WHO and ACSM on the importance of performing regular physical activity for at least 12 weeks of moderate intensity in a combined training modality (endurance and resistance training) to maximise cardiometabolic

health. Additionally, we first evidence the significant improvements in MetS risk factors of WC, SBP, DBP and TRG with exercise training on a usual diet of <12 weeks. In extension, the experimental chapters of this thesis implemented an 8-week HEFHIIT and the MedDiet lifestyle intervention aimed at exploring the feasibility on improving cardiometabolic health markers in physically inactive, overweight/obese post-menopausal women.

7.3.1 Body Composition

Mirroring the findings from the meta-analysis, we show that HEFHIIT for 8 weeks on a usual diet while maintaining caloric intake (Chapter 6) led to significant reductions in WC. These reductions signify decreases in visceral adiposity, implicated by the significant reductions in VAT. Moreover, combining the MedDiet with HEFHIIT was superior in improving body composition than HEFHIIT alone, indicated by reductions in weight, HC, BMI, body fat percentage and increases in lean mass (Table 7.1).

Interval training offers a time efficient way to improve body composition (Khodadadi et al., 2023), particularly in individuals with overweight and obesity. Previously published literature investigating HIIT interventions predominantly utilises treadmills and cycle ergometers (Batacan et al., 2017), which may be unreflective of real-world applications and may exclude individuals who lack accessibility to exercise facilities. WC is a surrogate marker for VAT, with every 1 cm increase in WC associated with a 5% increased risk in T2D (Marott et al., 2016). While there are no generally accepted reference values for VAT, the Tromsø Study in middle-aged adults of European origin found the VAT mass of ≥1134g to be the threshold for MetS prediction in women (Lundblad et al., 2021). Our study not only statistically reduced WC but also effectively decreased VAT, a key player in the progression of MetS and CMD. The reduction in abdominal adiposity is suggested to be attributed to HIIT's association with heightened catecholamine response, thus promoting lipolysis via β-adrenergic receptors especially in VAT (Zouhal et al., 2008).

Published literature have shown effectiveness of combined hypocaloric diet and physical activity in reducing abdominal/visceral adiposity. Similarly, our study demonstrates a contrast between Chapter 5 and 6, where statistical reductions in WC and VAT were absent following HEFHIIT in Chapter 5, but evident in Chapter 6. The disparity is likely underpinned to diet, as nutritional intake was not monitored in Chapter 5. Interestingly, dietary assessments from Chapter 6 revealed no changes in caloric intake, indicating that beneficial reductions in abdominal adiposity can occur even without calorie restriction.

While previous literature supports favourable weight loss and body compositional parameters with hypocaloric MedDiet and exercise (Konieczna et al., 2023), we show that combined HEFHIIT and MedDiet (EX+D) while maintaining baseline caloric intake significantly reduced abdominal obesity, weight and total body fat loss. Furthermore, when compared to HEFHIIT, the addition of the MedDiet induced greater total body fat percentage loss, strengthening the evidence of available literature in post-menopausal women (Khalafi & Symonds, 2023). Indeed, caloric restriction aids in weight loss by creating an energy deficit, while exercise alone increase EE.

However, combining both caloric restriction with exercise is more effective in reducing overall energy balance. Moreover, calorie restriction and exercise aids in better VAT reductions than exercise alone (Abe et al., 2021). While caloric restriction lifestyle interventions may be efficacious in reducing VAT, a caveat of adopting this approach is the lack of sustainability which can further contribute to the challenges of maintaining a healthier lifestyle. Similar to the existing literature, our findings show weight loss with the MedDiet without caloric restriction (Tussing-Humphreys et al., 2022). Moreover, alterations in macronutrients, such as increased olive oil and nuts in this study, may explain weight loss in the absence of caloric restriction (Sun et al., 2023). However, it is worth noting that nutritional data found no changes in macronutrient composition and dietary misreporting may have influenced these results (Ravelli & Schoeller, 2020). Thus, interpretations should be made with caution.

Notably, no concurrent increase in lean body mass was observed alongside VAT reduction, thus it remains inconclusive if the unchanged weight reflects body water retention or other factors due to the lack of available data. On the other hand, the EX+D group exhibited significant weight loss (-1.5 kg, -2%), although this was not clinically meaningful. It is important to acknowledge that while a reduction in 5% body weight has been reported to be associated with better cardiometabolic health in overweight/obese individuals, weight loss was not the primary emphasis of this thesis. The reductions in VAT in response to the interventions holds clinical relevance, as decreases in central adiposity is associated with subsequent reductions in mortality risk (Mulligan et al., 2019). This underscores the notion that while weight loss is commonly an emphasised outcome, focusing solely on this metric should not overshadow the potentially more clinically relevant benefits associated with VAT reductions achieved with both lifestyle interventions.

Table 7.1 Summary of results for body composition variables across all studies.

Body Composition			**WC (cm)**	**VAT (g);**	**Weight (kg)**	**BMI (kg/m^2)**	**HC (cm)**	**Body Fat (%)**	**Lean Mass (kg)**
Chapter 4 (MA)	ALL		**-2.62; <0.001**						
	<12 Weeks		**-2.18; 0.03**						
Chapter 5 (GHREX)	EX (n=10)	Effect; *P*	-1.6; 0.19	-15.8; 0.31	-0.4; 0.51	-0.3; 0.63	-1.0; 0.19	-0.7; 0.87	-0.2; 0.49
Chapter 6 (GHREXD)	CTL (n=10)	Effect; *P*	-0.4; 0.33	-76.7; 0.28	**1.2; 0.003**	**0.4; 0.011**	-1.3; 0.3	-0.8; 0.10	1.0; 0.06
	EX (n=10)	Effect; *P*	**-2.1; 0.04**	**-327.5; 0.002**	-0.6; 0.2	-0.3; 0.18	-0.5; 0.6	0.1; 0.9	-0.3; 0.6
	EX+D (n=10)	Effect; *P*	**-1.8; 0.005**	**-114.1; 0.01**	**-1.5; 0.005**	**-0.6; 0.004**	**-2.6; 0.03**	-2.4; 0.013	**0.9; 0.049**

Results for Chapter 4 (meta-analysis) reflects the effect sizes comparing mean change scores calculated from pre- and post-intervention. Results for Chapter 5 (GHREX) and Chapter 6 (GHREXD) represents the mean change scores calculated from pre- and post-intervention within group. Blue represents component of metabolic syndrome diagnosis. WC: waist circumference; VAT: visceral adipose tissue; BMI: body mass index; OW: overweight; OB: obese; HC: hip circumference.

7.3.2 Blood Pressure

In Chapter 6, both HEFHIIT with/without the MedDiet interventions led to significant reductions in DBP at the end of 8 weeks. Interestingly, in addition to the significant reductions in DBP, SBP significantly improved in Chapter 5, mirroring the results of our meta-analysis (Table 7.2). the disparity between the reductions in SBP between the HEFHIIT groups in both chapters could be attributed by the higher baseline SBP average of 131 mmHg in Chapter 5, compared to baseline 126 mmHg in Chapter 6. Mohr *et al.* reported that a reduction in SBP is a function of the higher-than-normal baseline values of SBP (Mohr et al., 2014), as seen in Chapter 5. While none of the participants were clinically hypertensive, SBP ≥130 mmHg contributes to MetS diagnosis. Thus, the clinically relevant reduction of -6 mmHg in SBP in Chapter 5 holds importance as a reduction of 5 mmHg in SBP is associated with a 14%, 9% and 7% reduction in stroke, coronary heart disease and all-cause mortality respectively.

Arterial pressure influenced by cardiac output, peripheral vascular resistance and arterial elasticity. The physiology of SBP refers to the maximum pressure of the arteries during left ventricular contraction (systole), and DBP refers to the arterial pressure during left ventricular relaxation (diastole) (Printz & Jaworski, 2018). Obesity-related mechanisms can elevate BP, leading to hypertension as previously discussed in section 2.2.4. Conversely, exercise can lower BP (Edwards et al., 2011). The antioxidative characteristics of the MedDiet can also improve BP by increasing endothelial dilating factors such as NO, leading to the lowering of peripheral vascular resistance (Psaltopoulou et al., 2004). HIIT (Li et al., 2022) and combined exercise with MedDiet interventions (Malakou et al., 2018) have consistently demonstrated significant reductions in both SBP and DBP, or isolated increases in SBP. Mechanisms behind improvements in BP with HIIT include increased cardiac output and enhanced oxygen utilisation due to increased muscular capillarisation (Yue et al., 2022). The mechanism behind isolated DBP decreases seen in Chapter 6 remains unclear, although exercise-induced improvements in peripheral vascular resistance through vasodilation of resistance vessels within the exercising skeletal muscle may play a role (Brett et al., 2000).

Reductions in BP with HEFHIIT with/without MedDiet may benefit the management of menopause-related symptoms. Oestrogen deficiency is associated with declines in endothelial function and BP (Moreau et al., 2012), contributing to with vasomotor symptoms (VMS); i.e. hot flushes, as well as elevated BP and CVD risk. Dysregulated autonomic nervous system (ANS), comprising both sympathetic and parasympathetic nervous systems, can lead to elevated BP and VMS in post-menopausal women (Lee et al., 2022). Exercise and diet can regulate ANS, offering the potential in the alleviation of VMS (Witkowski et al., 2023). However, evidence regarding mechanisms, optimal exercise prescriptions, and meaningful differences in VMS experiences are inconclusive. Future research is warranted to explore the effectiveness of exercise and dietary interventions in alleviating VMS in peri- and post-menopausal women.

Table 7.2 Summary of results for blood pressure across all studies.

Blood Pressure (mmHg)			SBP	DBP
Chapter 4 (MA)	ALL		**-5.95; <0.001**	**-4.14; <0.001**
	<12 Weeks		**-6.10; <0.001**	**-4.61; <0.005**
Chapter 5 (GHREX)	EX (n = 10)	Effect; *P*	**-6; 0.049**	**-4; 0.004**
Chapter 6 (GHREXD)	CTL (n = 10)	Effect; *P*	2.2; 0.45	0.9; 0.57
	EX (n = 10)	Effect; *P*	-0.4; 0.91	**-4.3; 0.027**
	EX+D (n = 10)	Effect; *P*	-3.6; 0.23	**-6.2; <0.001**

Results for Chapter 4 (meta-analysis) reflects the effect sizes comparing mean change scores calculated from pre- and post-intervention. Results for Chapter 5 (GHREX) and Chapter 6 (GHREXD) represents the mean change scores calculated from pre- and post-intervention within group. Blue represents component of metabolic syndrome diagnosis. SBP: systolic blood pressure; DBP: diastolic blood pressure.

7.3.3 Lipids

Following HEFHIIT with/without MedDiet in both experimental chapters, lipid profiles assessments revealed no changes at the end of 8 weeks. This contrasts with Chapter 4, where exercise interventions of <12 weeks resulted in significant reductions in TRG (Table 7.3).

As previously described in section 2.2, excess abdominal adiposity from elevated VAT plays a pivotal role in the pathophysiology of CMD. Excess VAT increases the release of FFA, thereby contributing to dyslipidaemia (Frayn et al., 2003). HIIT programs have been closely associated with improved oxidation capacity of fatty acids and favourable lipid profiles due to its association with increasing expression of key metabolic enzymes (Hausswirth et al., 2019). With the significant reductions in VAT observed in this study, it was interesting to observe the lack of association between reductions in VAT and lipid markers. The small sample size may have underpinned these findings.

Consensus from our and previous meta-analysis (Buzdagli et al., 2022) suggests that aerobic exercise training of <12 weeks significantly improves TRG, as well as TC. The possible mechanism behind exercise-induced improvement in lipid profiles involves the increased activity of enzymes such as lecithin-cholesterol acyltransferase (Calabresi & Franceschini, 2010) and lipoprotein lipase (LPL) (Kiwata et al., 2016), thus enhancing lipid utilisation by the skeletal muscles through fatty acid oxidation, thereby reducing plasma lipid levels (Muscella et al., 2020). Substrate usage during exercise is dependent on duration and intensity, with greater glucose utilisation during high intensity exercise, and fatty acid oxidation during moderate exercise (Chycki et al., 2019). This could be one of the possible explanations behind the statistically insignificant improvements in lipids profiles in this study. Yet, benefits of HIIT on improving lipid profiles, particularly among post-menopausal women are inconclusive. In response to 12 weeks of HIIT, one study reported no improvements in lipid profiles in post-menopausal women with MetS (Steckling et al., 2019), while another study reported significant reductions in TC and LDL in healthy post-menopausal women (Mandrup et al., 2017).

It has been previously reported that there is a linear dose-response relationship between exercise levels and HDL cholesterol levels, with training volume being a key determinant in improving lipid levels (Kraus et al., 2002), specifically a minimum EE of 1100 kcal of exercise to elicit significant increase in HDL (Ferguson et al., 1998). In accord with our meta-analysis, previous meta-analysis in adults report no significant effects of HDL with exercise (Buzdagli et al., 2022). Consistent with our findings in the experimental chapters, we did not observe statistical increases in HDL across all intervention groups in this thesis. Additionally, it was surprising to note the absence of statistically significant improvements in HDL in the EX+D. Olive oil, an important component of the MedDiet, contain polyphenols known to improve HDL anti-atherogenic function (Sarapis et al., 2023). Nevertheless, the increases in 0.1 mmol/L in HEFHIIT with/without MedDiet in Chapter 6 still hold importance as every 0.026 mmol/L increase in HDL corresponds to a 2 – 3% reduction in CVD prevalence (Gordon et al., 1989). It is posited that increased volume and longer commitment to both interventions will elicit greater improvements in HDL, translating to better risk reductions over time.

Table 7.3 Summary of results for lipid markers across all studies.

Lipid (mmol/L)			**TRG**	**HDL**	**TC**	**LDL**
Chapter 4 (MA)	ALL		**-0.4; 0.01**	**0.84; <0.001**		
	<12 Weeks		**-0.96; 0.007**	1.04; 0.05		
Chapter 5 (GHREX)	EX (n = 10)	Effect; *P*	-0.1; 0.65	0.0; 0.96	0.0; 0.99	0.0; 0.90
Chapter 6 (GHREXD)	CTL (n = 10)	Effect; *P*	0.0; 0.99	0.1; 0.45	0.4; 0.32	0.3; 0.31
	EX (n = 10)	Effect; *P*	0.1; 0.33	0.1; 0.28	0.3; 0.42	0.2; 0.57
	EX+D (n = 10)	Effect; *P*	0.0; 0.69	0.1; 0.36	0.3; 0.17	0.1; 0.49

Results for Chapter 4 (meta-analysis) reflects the effect sizes comparing mean change scores calculated from pre- and post-intervention. Results for Chapter 5 (GHREX) and Chapter 6 (GHREXD) represents the mean change scores calculated from pre- and post-intervention within group. Blue represents component of metabolic syndrome diagnosis. TRG: triglycerides; HDL: high-density lipoprotein; TC: total cholesterol; LDL: low-density lipoprotein.

7.3.4 Glycaemic Markers and Insulin Sensitivity

Chapters 5 and 6 measured fasting plasma glucose, HbA1c, insulin and markers of insulin sensitivity. We did not find significant changes in these markers in the intervention groups in both studies (Table 7.4). This was not unexpected as participants were within normal reference ranges for all markers. Furthermore, the lifespan of erythrocyte ranges from 90 – 120 days, explaining the net zero changes in HbA1c across the experimental studies.

The absence in FBG changes is in parallel with the findings of our meta-analysis with exercise interventions lasting <12 weeks. Interestingly, when participants were stratified according to FBG status in Chapter 5, we saw significant improvements in FBG by -0.5 mmol/L in 8 weeks. While this study consists of a small study sample, it sheds light on the potential of improving FBG with

HEFHIIT in post-menopausal women with dysglycaemia. The mechanism for exercise-induced improvements in FBG is owed to the role of skeletal muscle. During exercise, skeletal muscles promote GLUT-4, a key transporter, allowing glucose uptake independent of insulin. Therefore, accumulating bouts of exercise, will favour not only improvements in FBG, but also insulin sensitivity (Syeda et al., 2023).

Improvements in IR following HIIT interventions have been reported in overweight/obese adults (8 weeks) (De Matos et al., 2018) and post-menopausal women at risk of T2D (12 weeks) (Martins et al., 2018). Although statistically insignificant, individual improvements in insulin levels and markers of insulin sensitivity were observed in those at risk following HEFHIIT with/without MedDiet in this study. This is important as an increase in insulin sensitivity can reduce the incidence of age-related chronic conditions including hypertension and CVD. In expansion of exercise-induced increases of GLUT-4, the mechanisms proposed for improvements in insulin sensitivity has been owed to increase in muscle glycogen with HEFHIIT, thus enhancing insulin-stimulated glycogen synthesis (Burgomaster et al., 2008). Additionally, reductions in abdominal obesity can reduce circulating FFA and lipotoxicity, thereby improving insulin-stimulated glucose uptake, glucose oxidation and glucose synthesis (Boden, 2001).

Table 7.4 Summary of results for glycaemia and insulin variables across all studies.

Glycemia and Insulin			FBG (mmol/L)	HbA1c (%)	Insulin (mIU/L)	HOMA2-IR	HOMA2-%B	HOMA2-%S
Chapter 4 (MA)	ALL		**-0.38; <0.001**					
	<12 Weeks		0.79; 0.08					
Chapter 5 (GHREX)	EX (n=10)	Effect; *P*	0.1; 0.44	0.8; 0.84	0.6; 0.58	0.8; 0.84	-1.47; 0.87	-2.1; 0.71
Chapter 6 (GHREXD)	CTL (n=10)	Effect; *P*	0.2; 0.35	0.0; 0.99	**3.7; 0.018**	**0.5; 0.016**	17.3; 0.17	**-30.8; 0.009**
	EX (n=10)	Effect; *P*	0.1; 0.63	0.0; 0.52	-1.9; 0.12	-0.14; 0.15	-16.3; 0.06	3.1; 0.39
	EX+D (n=10)	Effect; *P*	0.2; 0.59	0.0; 0.57	-1.1; 0.39	-0.13; 0.43	-12.8; 0.34	-0.2; 0.51

Results for Chapter 4 (meta-analysis) reflects the effect sizes comparing mean change scores calculated from pre- and post-intervention. Results for Chapter 5 (GHREX) and Chapter 6 (GHREXD) represents the mean change scores calculated from pre- and post-intervention within group. Blue represents component of metabolic syndrome diagnosis. FBG: fasting blood glucose; HbA1c: glycated haemoglobin; HOMA2-IR: homeostasis model assessment 2 of insulin resistance; HOMA2-%B: homeostasis model assessment 2 of beta cell function; HOMA2-%S: homeostasis model assessment 2 of insulin sensitivity.

7.3.5 Cardiorespiratory Fitness

Here, we observed significant improvements of 2.9 ml/kg/min in estimated aerobic capacity following HEFHIIT. Mirroring our findings, Blackwell *et al.* (Blackwell et al., 2017) and Sian *et al.* (Sian et al., 2022) also found significant improvements in CRF with a commitment time of 37.5 minutes/week of HEFHIIT for 4 weeks. The physiological improvement in CRF is a strong indicator for reduced risk of all-cause mortality and cardiovascular morbidity (Laukkanen et al., 2016). An average increase of 3 – 4 mL/kg/min in aerobic capacity translates to an estimated risk reduction of 15% and 19% in all-cause and cardiovascular mortality (Lee et al., 2011), further supporting the cardiometabolic benefit of HEFHIIT.

The compliancy to the interventions in Chapter 6 were excellent, with 96% and 85% average completion of sessions in the HEFHIIT with/without the MedDiet, respectively. Interestingly, significant improvements in CRF were not observed with the addition of the MedDiet. Exercise compliancy is unlikely to be the factor behind the differences, considering that the average completed sessions were more than the HEFHIIT group. Previous reports show high adherence to the MedDiet to be associated with better CRF and BP in overweight/obese middle-aged adults, thus it is unclear of the explanation behind the findings of this study (Marcos-Pardo et al., 2020). Nevertheless, it should not be discounted that combined HEFHIIT and the MedDiet is still useful in improving CRF, evident from an increase in 1.6 mL/kg/min in aerobic capacity.

Given that fitness is one of the strongest independent predictors of overall health, these findings are significant. Enhanced CRF is linked to numerous health benefits beyond mortality reduction, including improved metabolic health, reduced inflammation, and enhanced mental well-being (Gray et al., 2015). The implications of our results suggest that HEFHIIT, even when performed independently or with the MedDiet, is a potent intervention for improving aerobic capacity and reducing cardiometabolic risk factors. This highlights the importance of promoting HEFHIIT as a viable, time-efficient exercise strategy to improve public health outcomes.

Future research should continue to explore the synergistic effects of dietary and exercise interventions, with a focus on long-term adherence and broader health impacts. Our findings contribute to the growing body of evidence supporting the use of HEFHIIT in various populations and emphasise the need for personalised approaches to optimise health interventions.

7.3.6 Risk Scores

While we have shown limited response in cardiometabolic-related blood markers from the experimental studies of this thesis, the utility of risk scores of VAI, MetS Z-score and QRISK3 serves as an insight into the quantitative assessment of multiple risk factors simultaneously on the effects of the intervention on risk profile. VAI accounts for WC, BMI, TRG and HDL into the equation, while MetS Z-score accounts for all the components of the MetS. The QIRKS3 score incorporates various risk factors including age, sex, BP, cholesterol levels and BMI to estimate an individual's 10-year risk of developing CVD.

There is a consistent increase in the number of participants that no longer meet the VAI threshold across the intervention groups at post-intervention. Furthermore, this improvement is also reflected in the MetS diagnosis criteria (Table 7.5). The clinical importance of VAI show that higher VAI scores are associated with significantly higher all-cause mortality in women (Tamosiunas et al., 2023). Moreover, MetS significantly increases risk of CMD as previously expanded in section 2.1.4. While the participants in this thesis were apparently healthy without previous diagnosis of MetS, it is clear that MetS risk prevails amongst healthy post-menopausal women, especially in those who are overweight or obese. While we saw reductions in risk for MetS criteria across the intervention groups, the risk reductions were not reflected by the MetS Z-scores. The MetS Z-score serves as a significant additional predictive value for CVD and mortality beyond the MetS

components, with its increasing value representing higher MetS severity (Honarvar et al., 2023). The disparity between the HEFHIIT groups of the experimental chapter could be underpinned by the inter-individual variability of training response to the intervention, with some individuals responding greater to exercise-induced cardiometabolic risk factors compared to others (Seward et al., 2019). This seems to hold true in this thesis, as we saw significant reductions in SBP in Chapter 5 that was absent in Chapter 6.

Although there were no statistical improvements in these risk scores, these subtle changes in these indices reflect positive shifts in underlying metabolic processes, evident from reductions in visceral adiposity and DBP. These are crucial determinants in the trajectory of maintaining cardiometabolic health, where these improvements through continuous efforts in sustaining these lifestyle behaviours may contribute to a cumulative effect over time, leading to clinically meaningful outcomes in the long term.

Table 7.5 Summary of results for risk scores across all studies.

Risk Scores; Cut-Off			VAI; >1.93 (Ahn et al., 2019)	MetS Z-score; >0	MetS; n	QRISK3 (%); ≥10%
Chapter 4 (MA)	ALL					
	<12 Weeks					
Chapter 5 (GHREX)	EX (n=10)	Effect; *P*	-0.15; 0.75	-0.08; 0.35	-2	-0.34; 0.10
Chapter 6 (GHREXD)	CTL (n=10)	Effect; *P*	-0.21; 0.44	0.03; 0.72	3; 0.25	0.1; 0.76
	EX (n=10)	Effect; *P*	0.04; 0.8	0.05; 0.65	-2; 0.69	-0.1; 0.69
	EX+D (n=10)	Effect; *P*	-0.1; 0.46	-0.02; 0.90	-2; 0.5	-0.3; 0.15

Results for Chapter 5 (GHREX) and Chapter 6 (GHREXD) represents the mean change scores calculated from pre- and post-intervention within group. VAI: visceral adiposity index; MetS: metabolic syndrome; QRISK3: cardiovascular risk score 3.

7.4 Declines in Cardiometabolic Profile Without Lifestyle Intervention

In contrast to the intervention groups, it is important to highlight that the absence of lifestyle intervention in the control group may have led to declines in cardiometabolic health over the same 8-week period. Evident through adverse changes such as weight gain, BMI and declines in insulin sensitivity, physical inactivity may have been contributed to pancreatic islet cell insufficiency, leading to IR as previously described in section 6.5.2.

While the weight gain and increase in BMI cannot be explained due to unchanged body composition or nutritional intake, it should be noted that short-term weight gain is associated with significantly increased odds of multi-morbidity in middle-aged women (Xu et al., 2019). Although the MetS Z-score did not change, we saw a gain in three participants from baseline that met the components for MetS diagnosis at the end of the 8 weeks. Without targeted lifestyle modifications, individuals in the control group may have continued to engage in behaviours associated with

metabolic dysfunction, such as unhealthy eating habits, sedentary lifestyle and inadequate physical activity, further exacerbating their cardiometabolic risk profile over time.

While the current guidelines recommend at least 75 minutes/week of vigorous physical activity, we were able to evidence improvements in more than one aspect of cardiometabolic health including abdominal obesity and DBP with either intervention, with a physical activity time commitment of 60 minutes/week in addition to their usual habitual physical inactivity. While the intervention groups may have not exhibited statistically significant improvements in risk scores, the comparison with the control group underscores the protective effects of incorporating either easily implementable lifestyle interventions in preventing the decline of cardiometabolic health.

7.5 Potential Ghrelin Mediated Pathway in Mitigating a Cardiometabolic Response?

While we failed to establish a relationship between the changes in ghrelin and cardiometabolic risk markers with either lifestyle interventions, the varying response of AG, DAG and weight loss to both lifestyle interventions brings us a step closer to understanding the multifaceted role of ghrelin function in improving cardiometabolic health.

In this thesis, we show that 8-week HEFHIIT in both Chapters 5 and 6 significantly increased fasting and post-prandial resting AG. It is acknowledged that the blood samples in both chapters were not identical, as blood collected in the former was post-prandial and the latter were fasting samples. Indeed, caloric state significantly influences circulating TG levels, with high and low levels during a fasted or fed state respectively. Nevertheless, given that obese individuals exhibit blunted AG levels in both fasted and post-prandial state (Wang et al., 2022), the increase in AG in both states suggest diminished ghrelin resistance with HEFHIIT. Ghrelin resistance arises from the impairment of ghrelin's functions in homeostatic feeding and reward processing due to positive energy balance, thus reducing ghrelin action in the brain (Zigman et al., 2016). As a signal of positive energy, elevated blood glucose in obese individuals stimulate insulin secretion that further suppresses ghrelin secretion, thus reducing circulating ghrelin levels (Yanagi et al., 2018). As a regulator of energy homeostasis (Al Massadi et al., 2017), the disruption in energy homeostasis in these overweight/obese individuals with HEFHIIT suggests an amelioration of ghrelin resistance through a compensatory increase in newly synthesised AG to protect the initial weight set point (Liang et al., 2021). This pattern, however, is contrasted in EX+D, where significant weight loss was seen that accompanied unchanged AG (and DAG). This suggests that while the increase in AG may be an indicator of increased ghrelin sensitivity in these obese individuals, the physiological role of AG may hinder weight changes. Moreover, due to the opposing effects of AG and DAG on appetite, insulin sensitivity and energy balance, the composition of TG from the balance of AG:DAG may offer a better insight of ghrelin-mediated cardiometabolic health.

A higher AG:DAG is reported to be associated with IR and increased body mass in obese individuals with MetS (Barazzoni et al., 2007). Exercise-induced weight loss is reported to accompany compensatory increased levels of TG, suggested to be driven by DAG (Ouerghi et

al., 2021). This is supported by improvements in body weight and body composition that was associated with an increase in DAG in young healthy men (Benso et al., 2012). In this study, it was unexpected to find no changes in DAG in the EX+D group, even with significant weight loss. The lack of studies investigating DAG response to lifestyle interventions in post-menopausal women makes it challenging to elucidate the mechanisms behind our findings. We theorise that restoration of ghrelin homeostasis of both isoforms driven by weight loss precedes restoration of cardiometabolic dysfunction. It is then through sufficient weight loss, where optimisation of ghrelin regulation may have downstream effects on cardiometabolic risk markers. In a recent study, improvements in insulin sensitivity were associated with TG changes in middle-aged adults following combined exercise and the MedDiet, although these results were only established in men (Tsaban et al., 2022). This research area is understudied but may have important implications for individualised medicine through optimisation of the ghrelin response with exercise with or without dietary intervention in post-menopausal women. Future research should involve longer duration studies to achieve clinical weight loss in the aid to understand if weight loss is a moderator of ghrelin-mediated cardiometabolic changes in post-menopausal women.

7.6 Future Perspectives

While investigation into predisposed physically inactive, overweight/obese post-menopausal women is warranted, exploration into pre- and peri-menopausal women as well may aid in better addressing menopause-related cardiometabolic disease progression. Longitudinal studies in women have shown that menopause is associated with the increased risk of MetS, independent of age (Christakis et al., 2020). Christakis *et al.* demonstrated that compared to pre-menopausal women, post-menopausal women had higher levels of HbA1c, TRG and SBP (Christakis et al., 2020). While menopause is inevitable, unravelling the complex changes and connections between menopause and metabolites may aid in potential targets to alleviate some of the downstream unfavourable menopause-associated health effects.

Cui *et al.* found distinct differences in metabolomic profile between pre- and postmenopausal women (Cui et al., 2019). Specifically, sphingomyelins and phosphatidylcholines were significantly higher in post-menopausal women. Both metabolites are components of plasma lipoproteins involved in lipoprotein assembly. The elevated levels of sphingomyelins and phosphatidylcholines is an indication of the increase in lipid profile associated with menopause, potentially related to weight gain and IR (Cui et al., 2019). While lifestyle modifications including HRT, exercise and diet may diminish CMD risks associated with the menopause, exploring their effects on individual metabolite markers will add a deeper level of understanding to better tailor menopause-related therapies. Higher habitual physical activity is associated with cardioprotective effects including better lipid profiles, lower FBG and leptin in post-menopausal women (Karvinen et al., 2019). Furthermore, the EMAS position statement also recommends the MedDiet to benefit menopause-related symptoms and health (Cano et al., 2020), due to its diet quality of higher-containing contents of including isoflavones, polyunsaturated fatty acids and phytochemicals.

Indeed, the use of HRT is effective against the implications of oestrogen deficiency evidenced through diminishing risks of impaired glucose tolerance and other aspects of MetS risk (Bermingham et al., 2022). However, the risks associated with HRT can outweigh the benefits. Furthermore, HRT use is not recommended past the age of 60 (Kim et al., 2020), where age-related comorbidities are more imminent. Collectively, these studies evidence the avenues available to improve the menopause transition and post-menopause period. However, the question of effectively mitigating and treating menopause-related health ailments on an individual level is still far from being answered. Future research into exploring the effects of these modifiable factors on metabolites across different cohorts of pre-, peri- and post-menopausal women may offer insights into the important opportunity to tailor preventative care and improve the health and longevity of women.

While this thesis aimed to shed light on utilising exercise and/or diet on ghrelin-mediated cardiometabolic health, it is crucial to acknowledge other pathways that influence ghrelin. In particular, the liver-expressed antimicrobial peptide 2 (LEAP2) has recently gained attention for its role in regulating appetite and energy balance (Mani et al., 2019). Produced in the liver, LEAP2 acts as a ghrelin antagonist, inhibiting ghrelin-induced food intake. As its fluctuations opposes to that of ghrelin, the LEAP2/ghrelin ratio serves as an indicator of energy status (Lu et al., 2021). Elevated levels of LEAP2 are found in individuals with obesity that negatively correlate with ghrelin levels (Mani et al., 2019). Furthermore, LEAP2 antagonises the insulinostatic effect of ghrelin (Hagemann et al., 2021). LEAP2 also respond acutely following certain nutrients. In rodents, plasma LEAP2 levels significantly increased following an acute dosing of olive oil (Gradel et al., 2023). Olive oil is a main component of the MedDiet and is plausible that the increased consumption contributed to the increase in LEAP2 and subsequent reductions in ghrelin isoforms. However, this does not explain the absence in AG or DAG changes post-intervention. Nevertheless, the investigation of LEAP2 is still in its preliminary stages. Its pathophysiological role and relationship with ghrelin warrants promising research in its role in modulating AG:DAG to benefit cardiometabolic health in post-menopausal women.

It should also be discussed that when considering HIIT for middle-aged sedentary post-menopausal women, it is essential to address relative risks, especially regarding occult CMD. While this demographic may not be at high risk during vigorous exercise, general risk mitigation strategies advocated by the ACSM, such as exercise supervision and gradual intensity progression, are crucial. Individualised risk assessment, pre-participation screening, and education on recognising cardiovascular distress symptoms are paramount. By prioritising safety and tailored care, HIIT can be effectively utilized to enhance cardiovascular fitness and cardiometabolic health in post-menopausal women, while minimising potential risks.

7.7 Methodological Limitations

The primary limitation of the present studies is the relatively small sample size, thus impeding robust inference of the outcome's statistical significance. The 8-week duration contributed to

participant exclusion, mainly due to lack of time. Furthermore, finding participants that were eligible presented another challenge, as majority were excluded due to being physically active. Another limitation was the absence of a 'MedDiet only' group that hindered the ability to isolate dietary effects from exercise. Furthermore, a follow-up visit could have aided in the understanding of adherence and behaviour beyond the intervention to establish the sustainability nature of the modalities. In Chapter 5 and 6, the use of incompatible ghrelin kits prevented a comprehensive analysis of ghrelin's relationship with the outcomes of interest. Addressing these limitations in future studies is essential to elucidate the modulation of AG:DAG on cardiometabolic health markers in post-menopausal women. Importantly, Chapter 5 lacked a control group, which limits the ability to attribute observed changes solely to the intervention rather than natural variation or other external factors. This should be considered when interpreting the results. Another significant limitation was the lack of standardisation of the HEFHIT protocol due to the use of self-chosen YouTube videos. This variability could have affected the consistency and comparability of the exercise interventions. Future studies should employ a standardised exercise protocol to ensure uniformity across participants. Finally, it should be noted that a small percentage (20% in Chapter 5, 17% in Chapter 6) of participants were on HRT. Indeed studies show that acute (21 days) (Kok et al., 2008) and long-term (6-month) (Kellokoski et al., 2005) treatment of HRT treatment elevate AG levels in menopausal women, thus participants in this thesis were recruited on the basis that they have been on HRT for at least 6 months for the metabolic effects of HRT to stabilise. While this does not discount the potentially effects of HRT on ghrelin, the results of the study should be minimally affected as no significant differences in baseline AG ($p = 0.62$) and DAG ($p = 0.77$) values between participants on or without HRT.

Despite the limitations, this thesis adds to the current evidence on the health benefits of home-based physical activity, particularly in abdominal obesity, in physically inactive, overweight/obese post-menopausal women. Furthermore, the addition of MedDiet to HEFHIIT aligns with the EMAS position statement on the cardiometabolic health benefit of MedDiet and menopausal health (Cano et al., 2020).

7.8 Conclusion

In conclusion, this thesis advocates the importance of regular exercise via feasible home-based, equipment-free high-intensity interval training (HEFHIIT) in benefitting abdominal obesity and diastolic blood pressure (DBP) in overweight/obese postmenopausal women. The addition of the Mediterranean-style diet to HEFHIIT further improves markers of cardiometabolic health, evidenced by favourable weight and total body fat loss. While we did not establish statistical improvements in other cardiometabolic blood markers, we saw emerging reductions in disease risk in individuals that presented at-risk thresholds at baseline. In contrast, the absence of targeted lifestyle changes may lead to declines in cardiometabolic health, evidenced from significant weight gain and declines in insulin sensitivity over the same duration. The high adherence rates show that these lifestyle behaviours can be easily implemented, and post-

menopausal women should not be discouraged from adopting these changes. Improvements in abdominal obesity, DBP and subtle improvements in other cardiometabolic blood markers are crucial determinants in the trajectory of maintaining cardiometabolic health, where continuous efforts in sustaining these lifestyle behaviours may contribute to a cumulative effect over time, leading to clinically meaningful outcomes in the long term.

While we saw significant increases in fasted and resting post-prandial acyl ghrelin (AG) following 8 weeks of HEFHIIT, we did not establish associations between exercise-induced ghrelin mediation and cardiometabolic changes. Exercise-induced weight loss is typically accompanied by augmented total ghrelin levels, thus it was proposed that greater weight loss is required to better understand ghrelin-mediated alterations in cardiometabolic health.

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Appendix 1 – GHREX Ethical Approval and Participant Facing Documents (Chapter 5)

Health Research Authority

West Midlands - Solihull Research Ethics Committee

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15 March 2021

Dr Rachel Churm
ASTEM, A127 Engineering East
Bay Campus, Swansea
University Swansea
SA1 8EN

Dear Dr Churm

Study title:	**Investigation of an exercise-induced alteration of the ghrelin axis and its implications in prediabetes.**
REC reference:	**21/WM/0045**
Protocol number:	**RIO 035-20**
IRAS project ID:	**288904**

Thank you for your letter of 15 March 2021. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 15 March 2021.

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Copies of materials calling attention of potential participants to the research [GHRex Recruitment Poster]	3	15 March 2021
IRAS Checklist XML [Checklist_15032021]		15 March 2021
Other [Response to REC]	2	15 March 2021
Participant consent form [GHRex ICF]	3	15 March 2021
Participant information sheet (PIS) [GHRex PIS]	3	15 March 2021

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Copies of materials calling attention of potential participants to the research [GHRex Recruitment Email]	2	16 February 2021
Copies of materials calling attention of potential participants to the research [GHRex Recruitment Poster]	3	15 March 2021
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Sponsor Insurance]		01 August 2020
GP/consultant information sheets or letters [GHRex GP Letter]	2	16 February 2021
Instructions for use of medical device [GHRex CGM PIS]	1	16 February 2021
Interview schedules or topic guides for participants [GHRex Participant Training guide]	2	16 February 2021
Interview schedules or topic guides for participants [GHRex Chester step test]	2	16 February 2021
IRAS Checklist XML [Checklist_15032021]		15 March 2021
Letter from funder [GHRex SDMF Award Letter]		06 February 2020
Letter from sponsor [Sponsor Letter]	1	22 January 2021
Other [Response to REC]	2	15 March 2021
Participant consent form [GHRex ICF]	3	15 March 2021
Participant information sheet (PIS) [GHRex PIS]	3	15 March 2021
REC Application Form [REC_Form_27012021]		27 January 2021
Research protocol or project proposal [GHRex Protocol]	2	16 February 2021
Summary CV for Chief Investigator (CI) [CI CV]		
Summary CV for student [Student CV]		
Summary CV for supervisor (student research) [Supervisor CV-Churm]		
Summary CV for supervisor (student research) [Cv supervisor-PRIOR]		
Summary CV for supervisor (student research) [CV supervisor-Bracken]		
Validated questionnaire [IPAQ]	2	16 February 2021
Validated questionnaire [Post Menopausal Questionaire]	2	16 February 2021

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

IRAS Project ID: 288904 **Please quote this number on all correspondence**

Yours sincerely

Sarah Graves
E-mail: solihull.rec@hra.nhs.uk

Swansea University
Prifysgol Abertawe

Participant Information Sheet

Investigation of an exercise-induced alteration of the ghrelin axis and its implications in prediabetes

Swansea University's Sports and Exercise department and the Medical School would like to invite you to take part in our research study that is being undertaken as part of an educational qualification. The study is jointly funded by the St David's Medical foundation and the College of Engineering in Swansea University.

Joining the study is entirely up to you, before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through this information sheet with you, to help you decide whether or not you would like to take part, and answer any questions you may have. Please feel free to talk to others about the study if you wish. **Part 1** of the Participant Information Sheet tells you the purpose of the study and what will happen to you if you take part. **Part 2** will give you more detailed information about the conduct of the study. Please take your time to decide whether or not you wish to take part.

If you do decide to take part and do ask if anything is unclear. You are free to withdraw at any time without explanation.

Thank you for reading this.

Part 1

What is the purpose of this study?

Postmenopausal women are at increased risk of metabolic stress. This can be due to two main factors; an increase in fat stored centrally within the abdomen and the loss of protective agents for your hearts health. These factors in addition to low levels of physical activity can have a negative effect on your health.

The way our body stores fat can be linked to poor blood sugar levels and can result in the development of diseases such as prediabetes. Exercise is shown to help lower blood sugar levels in prediabetes, and also improve heart health. However, it is not fully understood, the pathway in which increased training acts to have these beneficial effects within postmenopausal women.

We want to explore how this relationship can impact proteins within the stomach that can control fat storage and blood sugar usage.

This study will look at how exercise changes the way our body stores its fat to promote a health benefit, without the use of drugs. It will allow us to look into how the stomach proteins could work to help regulate blood sugar levels. This project has the goal of improving our knowledge of how exercise impacts human fat and why this is important in obesity and diabetes. The purpose is to improve our knowledge of exercise in relation to related stomach hormones and chemicals made within the fat of the abdomen and how these might be important in ill-health related to diabetes and the metabolic state.

Why have I been invited to take part?

You have been chosen because you are:

- A female aged between 45 and 65 years
- Postmenopausal (have not had your period for more than a year)
- Overweight or obese individual (body mass index (BMI) 25-35 kg/m^2)
    - (BMI is equal to body weight (kg) divided by height squared (m^2))
- Sedentary (not having had supervised or unsupervised exercise or other aerobic exercise for at least six months prior to the study)
- Generally well enough to exercise

And because you do not have/are:

- Premenopausal (have had your period within the last year)
- Abnormal electrocardiogram (ECG) - irregular heart function
- Current smokers
- Current or history of substance abuse and/or excess alcohol intake
- Type 1 or 2 Diabetes
- Heart disease
- Cancer
- Gastrointestinal disease e.g. inflammatory bowel disease or irritable bowel syndrome
- Kidney disease
- Liver disease
- Pancreatitis
- On hormone therapy for <6 months
- Prescribed anti-hypertensive or beta-blocker medication

Do I have to take part?

Your participation in this study is entirely voluntary. It is up to you to decide whether or not to take part. We will describe the study and go through this information sheet which you will keep. We will then ask you to sign a consent form to show you have agreed to take part. The original signed and dated copy will remain within a site file, at the research centre and you will be given a copy to take home. You are free to stop the research at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will I have to do?

If you agree to take part, you will then be asked to complete the consent form. A health screening will then be conducted to assess your eligibility. This involves giving a blood sample, an ECG, blood pressure and a simple exercise test.

Based at home, you will be asked to complete an 8-week high-intensity interval training (HIIT) programme using your own body weight. HIIT is a form of very vigorous exercise performed in short bursts of maximum effort, broken up with rest or light exercise. Vigorous intensity exercise makes you breathe hard and fast; you will not be able to say more than a few words without pausing for breath. Examples of very vigorous exercise includes running up the stairs, lifting heavy weights, or sprinting up hills. For this particular study, we will ask you to exercise according to a protocol (see below) which includes body weight exercises such as squats, lunges and burpees.

We will ask you to come into Swansea University at the start (week 1) and end (week 8) of your programme so we can take some measures from yourselves to assess how completing the programme has altered how the body stores fat. This includes the use of a dual-energy x-ray absorptiometry (DEXA) machine that uses low-level of X-ray radiation. The amount of radiation used is a much lower level of radiation than a standard X-ray. This means that a member of the team can stay in the scanning room with you during the scan. DEXA is a non-invasive, quick, and painless procedure that involves you lying on your back on a table. You are advised to wear light clothing, and to remove any clothes containing metal (such as zips, hooks, and buckles), as well as any metal jewellery.

During the scan, a large scanning arm will be passed over your body to measure your whole-body fat content. As the scanning arms is moved slowly over your body, a narrow beam of low-dose X-rays will be passed through the part of your body being examined. The scan usually takes about 8 minutes. 'DEXA Radiation Risk' under Part 2 of this Participant Information Sheet will provide more information on DEXA radiation.

In addition, throughout the 8 weeks you would be asked to wear a heart rate and accelerometer to monitor your daily activity during the duration of the intervention. You would also be asked to wear a continuous glucose monitoring (CGM) device during week 1 and week 8. Throughout the programme you will be in contact with the team that can deliver the training sessions and/or help with any queries you have.

Screening Visit

You will be invited to attend a screening visit at Swansea University where you will be interviewed by one of the researchers. You will be asked not to eat or drink anything (except water) for more than 8 hours before the visit. You will be asked to fill in an International Physical Activity Questionnaire (IPAQ) and a self-questionnaire to assess your menopausal status.

You will have your blood test (in which we will assess your blood glucose and lipid profile, height and weight, and blood pressure measurements taken. You will also have an ECG. This is a non-invasive test to look at the health of your heart.

You will also have to complete a simple exercise test. This is to evaluate the fitness of your heart. This involves stepping to a beat which will last for a maximum duration of 10 minutes. The test consists of a total of 5 stages, each lasting for 2 minutes which the speed will increase gradually. The test is terminated at 80% of your maximum heart rate (220 – your age) or when you have reached the end of stage 5.

The health screening should last no longer than 2 hours.

Study Visits – Week 1 and 8

You will be invited to attend 2 sessions at Swansea University: at the start (first day of week 1) and at the end (final day of week 8) of the exercise program so that we can take measures from yourselves to assess how completing the programme has altered how the body stores fat. You will have your fasting blood glucose, full lipid profile, height and body weight measurements taken.

You will also have your whole-body mass and fat percentage assessed via a DEXA scan machine. This machine is a non-invasive, quick, and painless procedure that involves you lying on your back on a table.

Following the scan, you will be given a standardised test meal and water. 30 minutes after consuming the meal, you will begin exercising under supervision according to your chosen exercise protocol (see Exercise Training Protocol Booklet). Blood samples will also be taken from you before, during and post exercise at T=0, 25, and 55 minutes.

You will be provided and asked to wear a continuous glucose monitoring (CGM) device during week 1 and week 8. This is a small device that you wear just under your skin. It measures your blood sugar levels continuously throughout the day. You will be taught and guided onto how to use your CGM device. The CGM will not inhibit your daily activities, nor exercise.

The research team will be available for support and contact throughout the course of the 8 weeks.

Exercise Intervention: Training Protocol

You will be required to participate in 3, 20-minute sessions per week of unsupervised home-based HIIT exercise for 8 weeks, in which you will follow your provided exercise protocol. The exercise protocol will gradually increase in intensity and will eventually involve 10 sets of repeated 1-minute bouts of high-intensity exercise, interspaced with 1-minute of rest. During the high-intensity bouts of exercise, you will be advised to achieve ≥80% of your predicted heart rate maximum. This will be reflected as at least 17 out of 20 on the scale of on your Rate of Perceived Exertion (RPE). This is further explained on your exercise protocol booklet. You will be able to see this on the heart rate device. The 1-minute intervals will compose of bodyweight exercises with no rest. These exercises are detailed in your exercise protocol booklet, ranging from low, medium and high impact upon which you will choose to complete, based on which fits your needs best. HIIT video material will also be supplemented to you to demonstrate the exercise movements in a safe manner.

During week 1, you are advised to complete four sets of intervals. In week 2, the number of sets to be completed will increase to 6 sets. In week 3 and 4, the number of sets to be completed will be increased to 8. By week 5-8, you would be encouraged to complete 10 sets of intervals until the end of the exercise intervention. A schematic table depicting the 8-week exercise schedule will be available on your exercise protocol booklet. At the end of every exercise session, you would be asked to send information related to your perceived exertion (according to the RPE scale) and exercise completed to the researcher via an encrypted messenger service (Whatsapp).

You will also be invited every fortnightly (week 2, 4 and 6) to a face-face or virtual supervised training session with a personal trainer to assess your ability, and to answer any potential questions you may have.

Will I get paid for participating?

There are no payments for taking part in this study.

What are the benefits of taking part?

The direct benefit from taking part in this study will be a metabolic health screening and improvements in health related to the study. Additionally, the information we get from this study might help to improve the treatment of people who are overweight and at risk of developing diabetes.

What if something goes wrong?

Participating in the study will not put you at any increased risk of anything going wrong. Part 2 of this sheet provides information on how to deal with any problems if they arise.

Will my taking part in this study be kept confidential?

Yes, however you will be invited to join a group message thread (WhatsApp) and participate in virtual group exercise sessions to maintain your morale over the 8-week period - this is 100% optional. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

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Part 2

What will happen if I do not wish to carry on with the study?

Your participation in this study is completely voluntary and you may refuse to participate and are free to withdraw from the study at any time. If you withdraw from the study, we will dispose of the samples and withdraw them from the research.

What if there is a problem?

Participating in the study should not put you at any increased risk of anything going wrong. If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions or telephone Dr Rachel Churm ([redacted]). If you remain unhappy and wish to complain formally, you can do this through the following contacts.

For data issues –

If you wish to raise a complaint on how we have handled your personal data or if you want to find out more about how we use your information, the data controller for this project will be Swansea University. The University Data Protection Officer provides oversight of university activities involving the processing of personal data, and can be contacted at the Vice Chancellors Office: dataprotection@swansea.ac.uk. Your personal data will be processed for the purposes outlined in this information sheet.

For Health issues –

Then please contact the local health boards, Health watchdog. SBU Community Health Council

First Floor, Cimla Hospital, Neath, SA11 3SU

Tel: [redacted]

Website: http://www.wales.nhs.uk/sitesplus/902/home

For Management issues –

If there is a problem arisen with the management of the study then please contact the Head of College, Prof Huw Summers:

Professor Huw Summers

College of Engineering, Swansea University

[redacted]

[redacted]

What are the possible disadvantages and risks of taking part?

In the event that we discover something about your health that you were unaware of, for example if your blood tests are abnormal, we would immediately inform you of this and inform your GP so that you can be referred to an appropriate specialist. If you require more urgent assessment we would arrange this for you immediately within the hospital.

Some of the procedures in this study, such as the recording of your weight, height, full body DEXA scan and blood pressure present no risk to you. Other procedures, such as taking blood samples, can cause mild discomfort. The risks of taking a blood sample include: slight discomfort when the needle is inserted and possible bruising and a localised infection. These procedures will only be carried out by trained professionals that have received local training in phlebotomy and care will be taken to minimise pain and discomfort from the procedure.

During the exercise training, you will feel sensations associated with exercise, but these will constitute no greater risk than normal exercise activities.

DEXA Radiation Risk

If you take part in this study you will have a whole-body scan by a DEXA machine. Some of these will be extra to those that you would have if you did not take part. These procedures use ionising radiation to form images of your body to provide our study with information on your body fat. Ionising radiation may cause cancer many years or decades after the exposure. We are all at risk of developing cancer during our lifetime. 50% of the population is likely to develop one of the many forms of cancer at some stage during our lifetime. Taking part in this study will add only a very small chance of this happening to you.

The amount of radiation used during a DEXA scan is less than 2 days' exposure to natural background radiation (NBR). By comparison, a flight to North America is equivalent to approximately a week's exposure to NBR, whilst a chest X-ray is equivalent to approximately 3 days' exposure to NBR.

How will my taking part in this study be kept confidential?

Any information and samples which are collected during this research will be kept strictly confidential. You will be given a unique identifying number and any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised. Some parts of your medical records and the data collected for the study may be looked at by authorised hospital staff to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

Involvement of the General Practitioner/Family doctor (GP)

It is a requirement that your GP is informed of your participation in this study. This is necessary so that your GP can look out for possible side effects and make better treatment decisions based on the knowledge of your participation in this study.

What will happen to any samples I give and will genetic tests be done?

We consider the blood samples you donate for the research to be a gift. This sample will be used to look how exercise changes influences fat storage and stomach proteins. No genetic tests for

disease risk will take place. Samples will be stored by the investigators in a locked freezer within a locked laboratory in Swansea University. The anonymised samples may be kept after the duration of this particular research study.

What will happen to the results of the research study?

The results of the study may be published in a medical journal and might be presented to other doctors and scientists with an interest in diabetes. If reports or publications are generated from the study you will not be identified in these. The results will also be used to look at the possibility of a larger more focused study involving the understanding on the role of exercise and its relationship with stomach proteins and fat storage.

Who is organising and funding the research?

This work is being organised between St David's Medical Foundation and College of Engineering Swansea University as part of a PhD.

Who has reviewed the study?

This study was reviewed by the Swansea University Research & Governance team. This study was given a favourable ethical opinion for conduct in the NHS by the Research Ethics Committee (REC).

Who can I contact for further information?

This Patient Information Sheet and the Informed Consent Form contain important facts which you should consider when deciding whether you are willing to take part in this study. If at any time you have any questions about the study, your rights as a research participant, a study related injury or side effects you should contact the team involved in the study; upon which they will be available by email and telephone.

Name: Dr Rachel Churm
Telephone: [redacted]
Email: [redacted]

Name: Abbigail Tan
Telephone: [redacted]
Email: [redacted]

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GHR-ex

Home-based high intensity interval training study

Outline of the training program:

You should train 3 times per week. These sessions can be on any day of the week, and a day's rest between sessions. You can complete each session wherever you want.

Training protocol table (weekly):

You are advised to follow these timings for your exercise sessions. However, you can adjust the intensity accordingly to suit your fitness.

Training Type	Week															
	1		2		3		4		5		6		7		8	
High intensity	4x	60s	6x	60s	8x	60s	8x	60s	10x	60s	10x	60s	10x	60s	10x	60s
Low intensity (recovery)	4x	4min	6x	2min 30s	8x	1min 30s	8x	1min 30s	10x	60s	10x	60s	10x	60s	10x	60s
Total Time:	20 mins		21 mins		20 mins		20 mins		20 mins		20 mins		20 mins		20 mins	

Table 1. **Weekly Training Protocol Table**

During each high-intensity interval, you should aim to achieve a heart rate equal to/above your heart rate goal. This can be monitored using your heart rate monitoring device.

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

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

Warm up and cool down consists of 5 minutes of these exercises chosen by yourself:

- Neck stretch
- Shoulder stretch
- Tricep stretch
- Pelvic stretch
- Quad stretch
- Ankle-holding stretch
- Arm, leg and torso stretch
- Arm rotations
- Torso rotations
- Hip rotations
- Marching on the spot
- Jogging on the spot
- Lunges stretch
- Jumping jacks (or modified)

Low impact exercise:

- Burpees with no jump
- Side squat walk
- Plank shoulder taps
- Plank to opposite toe touch
- Jumping jacks (modified)

Moderate impact exercise:

- Mountain climbers
- Butt kicks
- Squat + kick
- Squat to toe touch
- Plank up/down
- Lunges (no jumping)

High impact exercise:

- Burpees
- Jumping jacks
- Lunge jumps
- Squat jumps
- Plank jumps
- Twisting mountain climbers
- High knee jogging

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Rate of Perceived Exertion (RPE) Scale

You should aim for **17 – 19 on the RPE scale.*

Rating	*Perceived Exertion*
6	*No exertion*
7	*Extremely light*
8	
9	*Very light*
10	
11	*Light*
12	
13	*Somewhat hard*
14	
15	*Hard*
16	
17	*Very hard*
18	
19	*Extremely hard*
20	*Maximal exertion*

Table 2. **Rate of Perceived Exertion Scale**

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POST-MENOPAUSE QUESTIONNAIRE for: Investigation of an exercise-induced alteration of the ghrelin axis and its implications in prediabetes

These questions relate to menopause. We define menopause as beginning after you have had no menstrual cycles for ONE YEAR. You have been given this questionnaire because you have indicated that you are post-menopausal.

Name of Researcher: Dr Rachel Churm

Participant Identification Number for this trial:

1. What was the approximate date of your last menstrual period? _____
2. What age did your menstrual cycles first become irregular? _____
3. Are you post-menopausal? (Answer YES, if your last menstrual period was over one year ago?)
   YES **NO**
4. If post-menopausal, what age did you consider yourself post-menopausal? *write N/A if not applicable _____
5. Are you currently taking hormone replacement therapy (HRT)?

   ( ) YES, I am currently on HRT

   If yes, how long have you been taking HRT? _____

   ( ) YES, I have taken HRT but do not currently

   If yes, how long ago was this? _____

   ( ) NO, I do not and have never taken HRT

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

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Prifysgol Abertawe

International Physical Assessment Questionnaire (IPAQ) for: Investigation of an exercise-induced alteration of the ghrelin axis and its implications in prediabetes

Name of Researcher: Dr Rachel Churm

Participant Identification Number for this trial:

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous and moderate activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No *If answer is 'no', skip to PART 2: TRANSPORTATION*

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing upstairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ days per week

   ☐ No vigorous job-related physical activity ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ hours per day

   _____ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last **7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ days per week

   ☐ No moderate job-related physical activity ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

   _____ hours per day
   _____ minutes per day

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

   _____ days per week

   ☐ No job-related walking ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

   _____ hours per day

   _____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you travelled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

   _____ days per week

   ☐ No traveling in a motor vehicle ***Skip to question 10***

9. How much time did you usually spend on one of those days **travelling** in a train, bus, car, tram, or other kind of motor vehicle?

   _____ hours per day

   _____ minutes per day

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

    _____ days per week

    ☐ No bicycling from place to place ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

    _____ hours per day

    _____ minutes per day

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**

_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shovelling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home ***Skip to PART 4: RECREATION, SPORT AND LEISURE TIME***

PHYSICAL ACTIVITY

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**

_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

    _____ **hours per day**

    _____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

    _____ **hours per day**

    _____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

Appendix 2 – Supplementary Materials for Chapter 4

Database Search Strategies

PubMed

(postmenopaus*[Title/Abstract] OR post-menopaus*[Title/Abstract] OR older adults[Title/Abstract])

AND (exercise training[Title/Abstract] OR aerobic exercise[Title/Abstract] OR aerobic training[Title/Abstract] OR strength training OR resistance training OR physical training OR physical exercise OR exercise)

AND (metabolic syndrome[Title/Abstract] OR cardiometabolic disease[Title/Abstract] OR cardiometabolic syndrome[Title/Abstract] OR metabolic health[Title/Abstract] OR blood pressure[Title/Abstract] OR fasting glucose[Title/Abstract] OR glycaemia[Title/Abstract] OR glycaemic control[Title/Abstract] OR lipid profile[Title/Abstract] OR waist circumference[Title/Abstract] OR anthropometry[Title/Abstract])

NOT (animals)[Title/Abstract]

Web of Science

Article title, Abstract, Keywords: (postmenopausal OR post-menopausal OR older women) AND (exercise training OR aerobic exercise OR aerobic training OR strength training OR resistance training OR physical training OR physical exercise OR exercise) AND (metabolic syndrome OR cardiometabolic disease OR cardiometabolic syndrome OR metabolic health OR blood pressure OR fasting glucose OR glycaemia OR glycaemic control OR lipid profile OR waist circumference OR anthropometry)

Scopus

Article title, Abstract, Keywords: (postmenopausal OR post-menopausal OR older women) AND (exercise training OR aerobic exercise OR aerobic training OR strength training OR resistance training OR physical training OR physical exercise OR exercise) AND (metabolic syndrome OR cardiometabolic disease OR cardiometabolic syndrome OR metabolic health)

Cochrane

#1 (“postmenopausal” OR “post-menopausal” OR “older women”): ti, ab, kw
#2 (“exercise training” OR “aerobic exercise” OR “aerobic training” OR “strength training” OR “resistance training” OR “physical training” OR “physical exercise” OR “exercise”): ti, ab, kw
#3 (“metabolic syndrome” OR “cardiometabolic disease” OR “cardiometabolic syndrome” OR “metabolic health”): ti, ab, kw

Supplementary Material Table S1. Risk of bias table presenting judgements based on assessment algorithms for Cochrane risk of bias tool for randomised trials (RoB2) for each risk of bias aspects for evaluation of quality for all 40 studies. Abbreviations: ITT – intention-to-treat, mITT – modified intention-to-treat, NA – not applicable

Cochrane RoB2 domain	Domain-level judgements	Reasons for judgements
(1) Bias arising from the randomization process	Low risk	Available description of random sequence generation and allocation concealment (in the manuscript or trial protocol) as well as no evidence for baseline imbalances.
	Some concerns	Missing description of allocation concealment, but no evidence for baseline imbalances.
	High risk	Missing description of allocation concealment but there is evidence that baseline imbalances were due to failure to proper random allocation of participants.
(2) Bias due to deviations from the intended interventions	Low risk	Study used ITT (or mITT) approach and reported high adherence of participants to exercise programs (i.e ≥80% of session completed)
	Some concerns	Study used ITT (mITT) or per-protocol approach and reported moderate non-adherence of participants to exercise program (i.e. rates not equal between groups)
	High risk	Study used per-protocol approach in case of serious non-adherence or unwillingness to continue exercise program.
(3) Bias due to missing outcome data	Low risk	Study's drop-out rate ≤20%, with stated reasons, and dropouts were likely not dependent on their true value.
	Some concerns	Study's drop-out rate >20%, but reasons for dropouts missing, and dropouts were likely not dependent on their true value
	High risk	Study's drop-out rate >20%, and dropouts were likely dependent on their true value (i.e., rates not equal between groups).

(4) Bias in the measurement of the outcome	Low risk	Measurements of outcomes stated clearly (i.e. waist circumference measured at the mid-point between the lowest rib and the iliac crest, with measurement made at the end of a normal expiration. Pre- and post-intervention blood measurements (blood glucose, HDL-C, TG) taken after an overnight fast. Blood pressure to be taken after at least 10 minutes of rest)
	Some concerns	Measurements of outcomes vaguely mentioned (i.e. waist circumference measurements not clearly stated)
	High risk	N/A
(5) Bias in the selection of the reported result	Low risk	Pre- and post-intervention values reported for both intervention and control groups
	Some concerns	Only change mean values (pre- and post-intervention) for both intervention and control groups
	High risk	Change mean values between intervention group and control group only

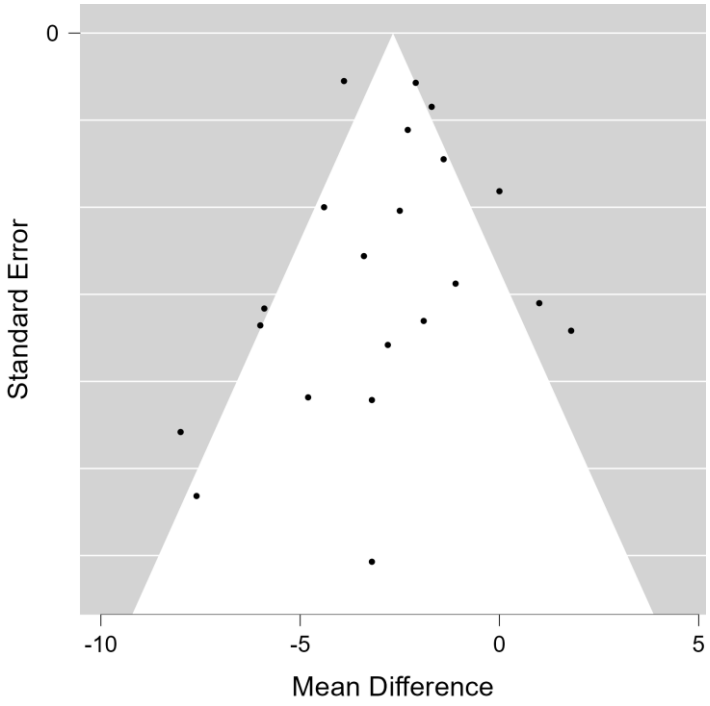
Supplementary Material Table S2. Risk of bias table summarised for all included studies (n=40). Aspects of RoB2 1 – 5 are elaborated in Table S1. [a,b] denotes sub-studies; FBG: fasting blood glucose; HDL: high-density lipoprotein; TRG: triglycerides; SBP: systolic blood pressure; DBP: diastolic blood pressure.

	FBG	HDL	TRG	SBP	DBP	WC	1	2	3	4	5
Akwa et al., 2017	✗	✓	✓	✓	✓	✗	?	?	+	+	+
Azadpour et al., 2017	✗	✗	✗	✓	✓	✓	?	?	+	+	+
Bergström et al., 2008	✗	✓	✗	✓	✓	✓	?	?	?	+	+
Biteli et al., 2021[a]	✓	✓	✓	✗	✗	✓	+	?	+	?	+
Biteli et al., 2021[b]	✓	✓	✓	✗	✗	✓	+	?	+	?	+
Chagas et al., 2017	✓	✓	✓	✗	✗	✓	?	?	+	?	+
Church et al., 2007a	✓	✓	✓	✓	✓	✓	+	+	+	+	?
Colado et al., 2009	✓	✓	✓	✓	✓	✓	?	?	+	?	+
Conceição et al., 2013	✓	✓	✓	✓	✓	✓	?	?	+	+	+
Dalleck et al., 2009[b]	✓	✓	✓	✓	✓	✓	?	+	?	+	+
Figueroa et al., 2011	✗	✗	✗	✓	✓	✗	?	?	+	+	?
Frank et al., 2005	✓	✗	✓	✗	✗	✗	?	+	+	+	?
Friedenreich et al., 2011	✗	✗	✗	✗	✗	✓	+	+	+	?	?
Gómez-Tomás et al., 2018	✗	✓	✓	✗	✗	✓	?	?	?	+	+
Hettchen et al., 2021	✓	✓	✓	?	?	✓	?	+	?	?	+
Jaime et al., 2019	✗	✗	✗	✓	✓	✗	+	+	+	+	+
Keyhani et al., 2020	✗	✓	✓	✓	✓	✗	?	?	+	+	+
Kim and Kim, 2012	✓	✓	✓	✓	✓	✓	?	?	+	+	+
Latosik et al., 2014	✗	✓	✓	✓	✓	✓	?	?	?	?	+
Lee et al., 2012	✓	✓	✓	✓	✓	✓	?	?	+	+	+
Lee et al., 2021	✗	✓	✓	✗	✗	✗	?	?	+	+	+
Lesser et al., 2016	✓	✗	✗	✗	✗	✓	?	?	+	+	+
Libardi et al., 2012	✗	✓	✓	✗	✗	✗	?	+	+	+	+
Marcus et al., 2009	✗	✗	✗	✗	✗	✓	?	+	+	+	+
Miyaki et al., 2012	✗	✓	✓	✓	✓	✗	?	?	+	+	+
Moreau et al., 2001	✓	✗	✗	✓	✓	✗	?	?	?	+	?
Neves et al., 2017	✓	✗	✓	✗	✗	✗	?	+	?	+	?
Nunes et al., 2016	✗	✓	✓	✗	✗	✓	?	+	+	+	+
Rezende Barbosa et al., 2019	✗	✗	✗	✓	✓	✗	+	?	?	+	+
Sénéchal et al., 2012	✓	✓	✓	✓	✓	✓	?	?	?	+	+
Seo et al., 2010	✓	✓	✓	✓	✓	✓	?	?	+	+	+
Son et al., 2017	✗	✗	✗	✓	✓	✗	+	?	+	?	?
Son and Park, 2021	✓	✓	✓	✓	✓	✓	?	?	?	+	?
Staffileno et al., 2001	✗	✗	✗	✓	✓	✗	?	?	?	+	+
Trabka et al., 2014	✗	✓	✓	✗	✗	✓	+	+	+	?	+
van Gemert et al., 2015	✓	✗	✗	✗	✗	✗	?	?	+	?	?
Ward et al., 2020	✗	✓	✓	✗	✗	✗	?	?	?	+	+

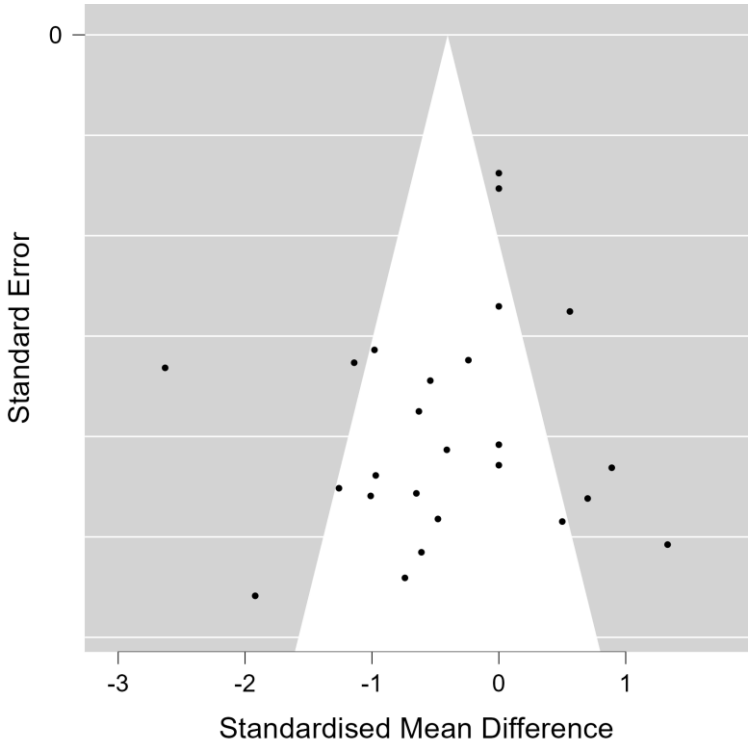
Wong et al., 2018	✗	✗	✗	✓	✓	✗	+	+	+	+	+
Wong et al., 2018	✗	✗	✗	✓	✓	✗	+	+	+	+	+
Wooten et al., 2011	✗	✓	✓	✗	✗	✗	?	?	+	+	+

Supplementary Material Figure S1. Funnel plots depicting the association of exercise training and metabolic syndrome risk variables. (a) waist circumference; (b) triglycerides; (c) HDL; (d) fasting blood glucose; (e) systolic blood pressure; (f) diastolic blood pressure.

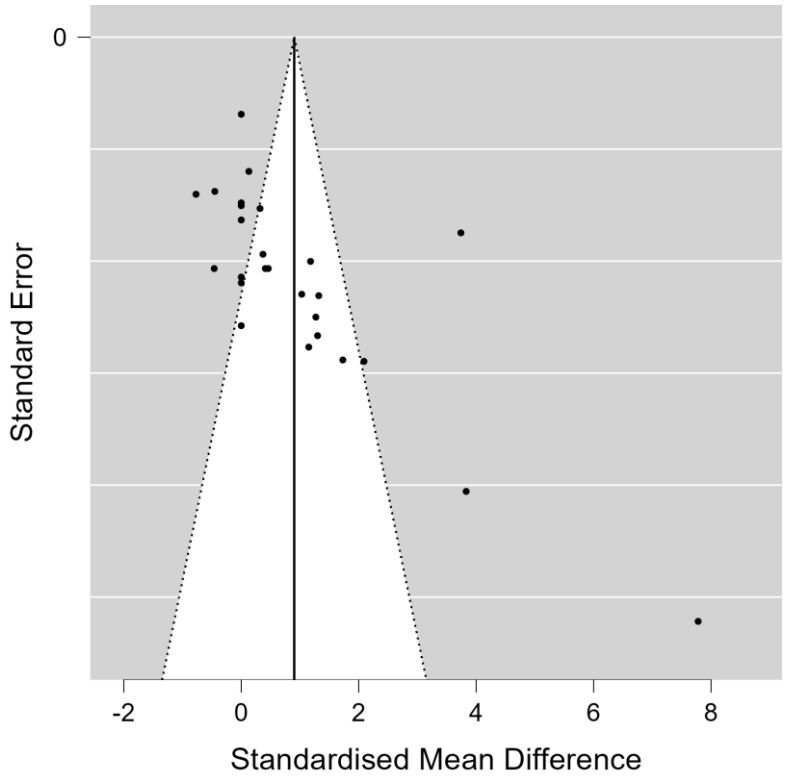
(a) WC: P = 0.157



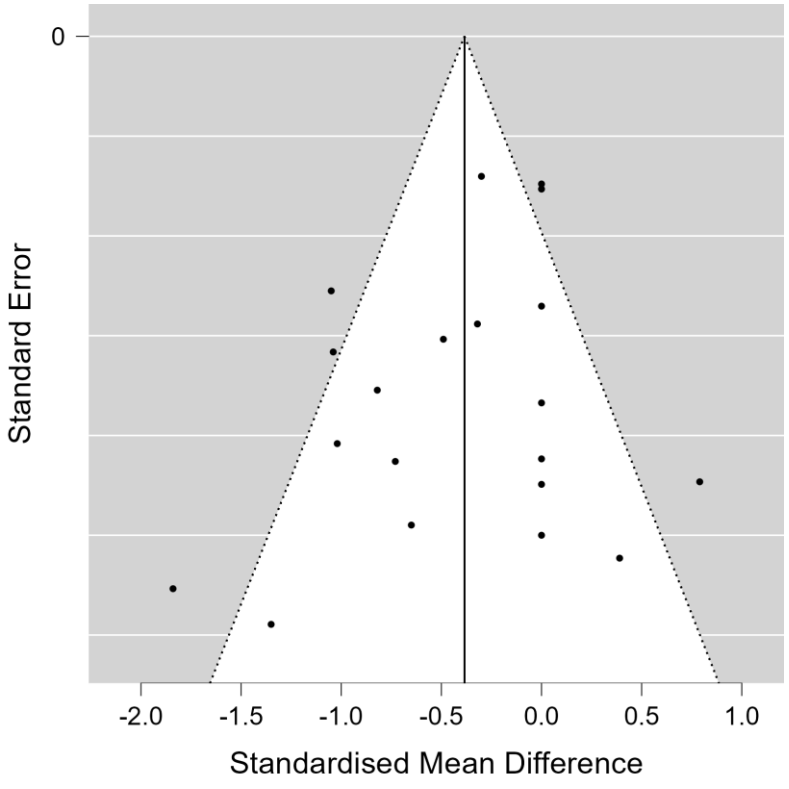
(b) Triglycerides: P = 0.688



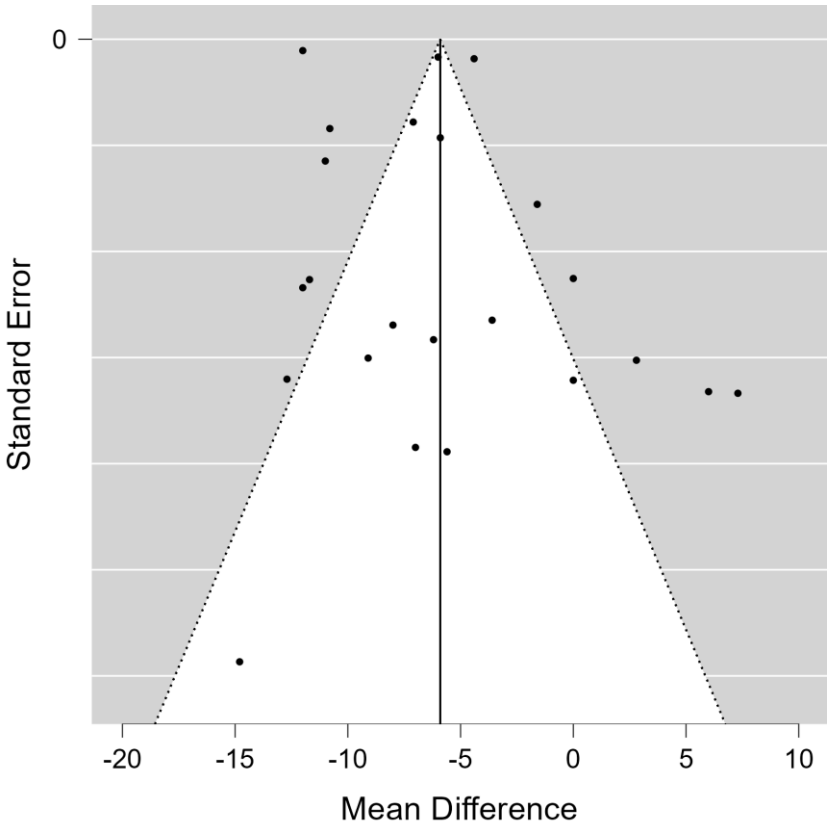
(c) HDL: P < 0.001



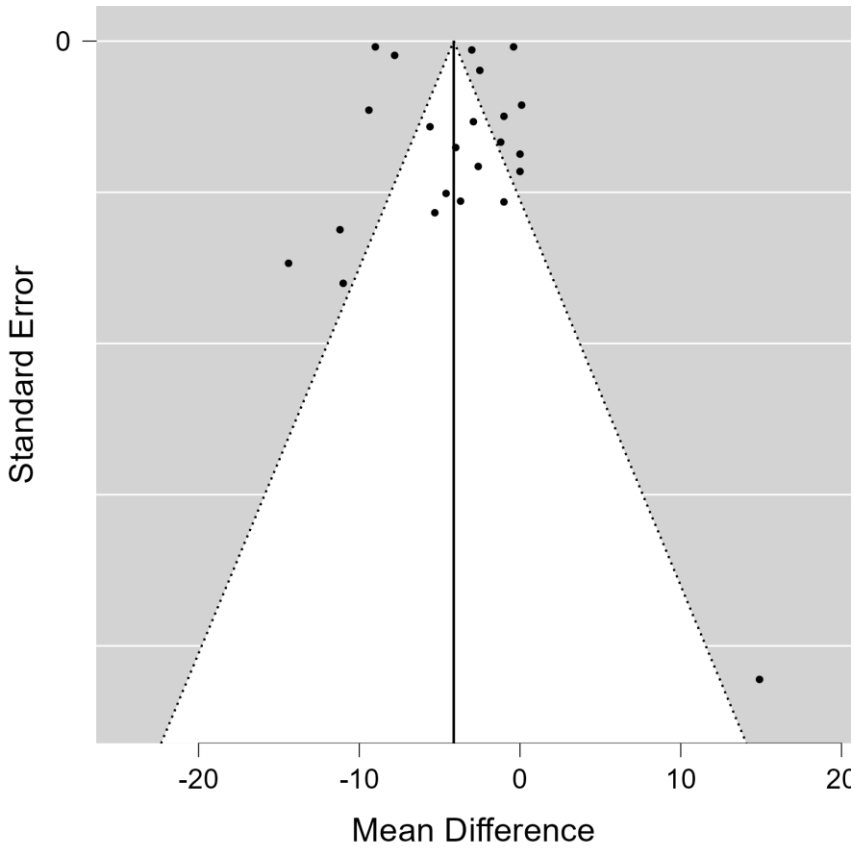
(d) Fasting Blood Glucose: P = 0.320



(e) SBP: P = 0.316



(f) DBP: P = 0.826



Appendix 3 – GHREXD RCT Ethical Approval and Participant Facing Documents (Chapters 6)

Health Research Authority

London - Hampstead Research Ethics Committee
Ground Floor
Temple Quay House
2 The Square
Bristol
BS1 6PN

20 April 2022

Miss Soon Ying Abbigail Tan
ASTEM, A127 Engineering East
Bay Campus, Swansea University
SA1 8EN

Dear Miss Tan,

Study title:	**Evaluation of lifestyle modification on cardiovascular risk in overweight and obese postmenopausal women: a ghrelin-mediated response**
REC reference:	**22/LO/0301**
Protocol number:	**RIO 013-22**
IRAS project ID:	**314505**

Thank you for your letter of 20 April 2022. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 19 April 2022

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Other		
Participant consent form [GHRexD ICF]	3	19 April 2022
Participant information sheet (PIS) [GHRexD PIS]	3	19 April 2022

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Copies of materials calling attention of potential participants to the research	1	21 March 2022
Copies of materials calling attention of potential participants to the	1	21 March 2022

research [GHRex Recruitment Email]		
Covering letter on headed paper		
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		
Letter from sponsor		
Other [Application Clarification]		30 March 2022
Other		
Participant consent form [GHRexD ICF]	3	19 April 2022
Participant information sheet (PIS) [GHRexD PIS]	3	19 April 2022
REC Application Form [REC_Form_29032022]		29 March 2022
Research protocol or project proposal [GHRexD Protocol]	2	25 March 2022
Summary CV for Chief Investigator (CI)		
Summary CV for student		
Summary CV for supervisor (student research) [Professor Bracken]		
Summary CV for supervisor (student research) [Dr Prior]		
Validated questionnaire [International Physical Activity Questionnaire (IPAQ)]		
Validated questionnaire [MEDITERRANEAN DIET SCORE TOOL]		
Validated questionnaire [Mediterranean Diet Booklet]		
Validated questionnaire [MENOPAUSE SYMPTOMS QUESTIONNAIRE]		
Validated questionnaire [Exercise Graph]		
Validated questionnaire [POST-MENOPAUSE QUESTIONNAIRE]	1	21 March 2022

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

IRAS Project ID: 314505 **Please quote this number on all correspondence**

Yours sincerely,

[redacted]

Kamile Janusonyte
Approvals Administrator

E-mail: hampstead.rec@hra.nhs.uk

Copy to: Miss Soon Ying Abbigail Tan

Lead Nation: Wales: research.permissions@wales.nhs.uk

Swansea University
Prifysgol Abertawe

Participant Information Sheet

EVALUATION OF LIFESTYLE MODIFICATION ON CARDIOVASCULAR RISK IN OVERWEIGHT AND OBESE POSTMENOPAUSAL WOMEN: A GHRELIN-MEDIATED RESPONSE.

Swansea University's Sports and Exercise department and the Medical School would like to invite you to take part in our research study that is being undertaken as part of an educational qualification. The study has an associate funding with the St David's Medical Foundation and the College of Engineering in Swansea University.

Joining the study is entirely up to you, before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through this information sheet with you, to help you decide whether or not you would like to take part, and answer any questions you may have. Please feel free to talk to others about the study if you wish. **Part 1** of the Participant Information Sheet tells you the purpose of the study and what will happen to you if you take part. **Part 2** will give you more detailed information about the conduct of the study. Please take your time to decide whether or not you wish to take part.

If you do decide to take part and do ask if anything is unclear. You are free to withdraw at any time without explanation.

Thank you for reading this.

Part 1

What is the purpose of this study?

Postmenopausal women are at increased risk of metabolic stress. This can be due to two main factors; an increase in fat stored centrally within the abdomen and the loss of protective agents for your heart's health. These factors in addition to low levels of physical activity can have a negative effect on your health. The way our body stores fat can be linked to poor blood sugar levels and can result in the development of diseases such as diabetes. Lifestyle modifications like exercise and diet have shown to improve aspects of health, such as weight management, blood sugar levels, and heart health. However, it is not fully understood, the pathway in which

increased levels in exercise training with or without diet control acts to have these beneficial effects within postmenopausal women. We want to explore how this relationship can impact proteins within the stomach that can control fat storage and blood sugar usage, as well as protection against heart and metabolic diseases.

This study will look at how exercise with or without the Mediterranean diet changes the way our body can promote heart and metabolic health benefits through the pathway of stomach proteins, without the use of drugs. This project has a goal of improving our knowledge of how we can use lifestyle modifications as an alternative treatment against conditions such as type 2 diabetes and obesity. The purpose is to improve our knowledge of exercise with or without the Mediterranean diet in relation to related stomach proteins and pathway-related chemicals in our body, and how these might be important in ill-health related to diabetes and the metabolic state.

Why have I been invited to take part?

You have been invited because you are:

- A female aged between 45 and 65 years
- Postmenopausal (have not had your period for more than a year)
- Overweight or obese individual (body mass index (BMI) 25-35 kg/m^2)
    - (BMI is equal to body weight (kg) divided by height squared (m^2))
- Physically inactive (IPAQ score – category I and not engaged in at least 60 min/week of structured exercise during the previous 6 months)
- Generally well enough to exercise (i.e. pass screening criteria)

Because you do not have:

- An abnormal electrocardiogram (ECG) - irregular heart function
- A current or history of substance abuse and/or excess alcohol intake
- Type 1 or 2 Diabetes
- Heart disease
- Cancer
- Gastrointestinal disease e.g. inflammatory bowel disease or irritable bowel syndrome
- Kidney disease
- Liver disease
- Pancreatitis

And are not:

- Premenopausal (have had your period within the last year)
- A current smoker
- On hormone therapy for <6 months
- Prescribed anti-hypertensive or beta-blocker medication

However, the results of the screening may indicate that you will not be invited to take part. We aim to recruit a minimum of 36 participants in total.

Do I have to take part?

Your participation in this study is entirely voluntary. It is up to you to decide whether or not to take part. You are encouraged to discuss this study with your family and friends before deciding

on whether to take part in this study. We will describe the study and go through this information sheet which you will keep. We will then ask you to sign a consent form to show you have agreed to take part. If you have any questions or worries, please do not hesitate to ask one of the research team members. The original signed and dated copy will remain within a site file, at the research centre and you will be given a copy to take home. You are free to stop the research at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will I have to do?

If you agree to take part, you will then be asked to complete the consent form. A health screening will then be conducted to assess your eligibility. This involves having your body measurements taken (height, weight, waist and hip circumference), an ECG, blood pressure and a simple exercise test.

This is an 8-week study, and you will be with us for 9 weeks. Depending on which group you will be assigned to at random, you will either complete 1) home-based exercise only program; 2) home-based exercise and Mediterranean diet program; 3) a control (no intervention). If you have been assigned to the intervention groups, you will complete an 8-week high-intensity interval training (HIIT) programme using your own body weight. HIIT is a form of very vigorous exercise performed in short bursts of maximum effort, broken up with rest or light exercise. Vigorous intensity exercise makes you breathe hard and fast; you will not be able to say more than a few words without pausing for breath. Examples of very vigorous exercise includes running up the stairs, lifting heavy weights, or sprinting up hills. For this study, we will ask you to exercise with reference to a protocol provided which includes body weight exercises such as squats, lunges and burpees. If you have been assigned to the exercise and Mediterranean diet group, you will adhere to a Mediterranean diet during the 8 weeks along with performing your home-based HIIT exercise. A member of the research team will introduce and brief you to the diet prior to the intervention. The Mediterranean diet encompasses a variety of plant-based foods and whole grains, moderate consumption of cheese, yoghurt, fish, poultry, and low consumption of red/processed meat and alcohol with a focus of utilising olive-oil as the main source of fat.

On top of the initial screening visit at Week 0, we will ask you to come into Swansea University at the start (week 1) and end (week 8) of your programme so we can take some measures from yourselves to assess how completing the programme has altered how the body stores fat. This includes the use of a dual-energy x-ray absorptiometry (DEXA) machine that uses low-level of X-ray radiation. The amount of radiation used is a much lower level of radiation than a standard X-ray. This means that a member of the team can stay in the scanning room with you during the scan. DEXA is a non-invasive, quick, and painless procedure that involves you lying on your back on a table. You are advised to wear light clothing, and to remove any clothes containing metal (such as zips, hooks, and buckles), as well as any metal jewellery.

During the scan, a large scanning arm will be passed over your body to measure your whole-body fat content. As the scanning arms is moved slowly over your body, a narrow beam of low-dose X-rays will be passed through the part of your body being examined. The scan usually takes about 8 minutes. ‘DEXA Radiation Risk’ under Part 2 of this Participant Information Sheet will provide more information on DEXA radiation.

Regardless of the group you have been assigned to, throughout the 8 weeks you will be asked to wear a heart rate and accelerometer to monitor your daily activity during the duration of the intervention. You will also be asked to record your daily foods consumed using an app called MyFitnessPal.

Throughout the programme you will be in contact with the team that can deliver the training sessions, the diet and/or help with any queries you have.

Screening Visit (Week 0)

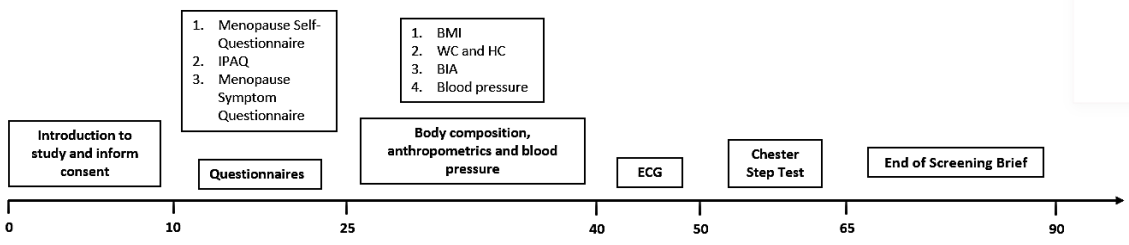
You will be invited to attend a screening visit at Swansea University where you will be interviewed by one of the researchers. You will be asked not to eat or drink anything (except water) from 8pm the night before your visit. If you wish to participate, you will be asked to sign a consent form. You will then be asked to fill in three questionnaires: 1) International Physical Activity Questionnaire (IPAQ); 2) self-questionnaire to assess your menopausal status; and 3) menopause symptoms questionnaire.

Body composition and body measurements will be taken. This involves your height, weight, waist and hip circumference. To assess your body composition measurements such as your body fat percentage and lean mass, a non-invasive test utilising a machine called the bio-electrical impedance analysis (BIA) will send a low-level, painless, electric current throughout your body. You will also have your blood pressure and an electrocardiogram (ECG) taken. An ECG is a non-invasive test to look at the health of your heart. During the ECG we will need to have access to your chest to place sticky sensors called electrodes. We suggest wearing a loose-fitting top or you may need to remove clothing (i.e. top) to allow for sensor placement. The electrodes will then be connected to a machine that will record your hearts rhythm.

You will also have to complete a simple exercise test. This is to evaluate the fitness of your heart. This involves stepping to a beat which will last for a maximum duration of 10 minutes. The test consists of a total of 5 stages, each lasting for 2 minutes which the speed will increase gradually. The test is terminated at 80% of your maximum heart rate (220 – your age) or when you have reached the end of stage 5.

If you are eligible to participate in the study, the researcher will disclose which group you will be assigned to. The allocation sequence of which you will be assigned to is at random (generated through computerised random sequence) and pre-defined. Please note that you are unable to change groups once assigned as this will create a bias within the study.

The health screening should last no longer than 2 hours. Below is a schematic of the screening visit at Week 0.



Study Visits – Week 1 and 8

You will be invited to attend 2 sessions at Swansea University: at the start (first day of week 1) and at the end (final day of week 8) of the exercise program so that we can take measures from yourselves to assess how completing the programme has induced changes in your body. Identical to your screening visit, you will have your blood pressure, body composition and measurements taken again. In additional, a fasting blood sample will be obtained via venepuncture (from your vein) and capillary blood sample (from your fingertip). Blood obtained will be used to analyse gene expression markers related to the ghrelin pathway, circulating concentrations of inflammatory markers, lipid levels (total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides levels), lactate, free fatty acids, acyl and des-acyl/total ghrelin, glucose, HbA1C and insulin. A total of 15 mL of blood (approximately 3 teaspoons) will be collected during each study visit.

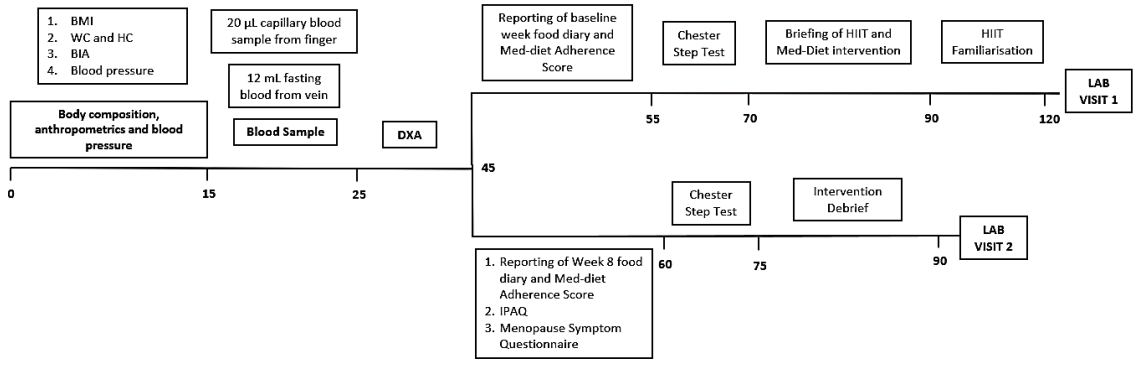
You will also have your whole-body mass and fat percentage assessed via a DEXA scan machine. This machine is a non-invasive, quick, and painless procedure that involves you lying on your back on a table.

Following the DEXA scan, you will perform the exercise test again as you did during your screening visit to reassess your fitness of your heart. If you have been allocated to the exercise and Mediterranean diet group, you will discuss your previous week's food diary and complete a Mediterranean diet adherence score form. The laboratory visit 1 will end with a mock exercise session with the researcher (if you have been allocated to either intervention groups) and a briefing to the 8-week intervention.

You will be provided and asked to wear an accelerometer during the entire 9 weeks except for charging the device. This allows the research team to compare your physical activity levels before and after, as well as capture as much physical and heart rate data during your time with us. The accelerometer can be worn in the shower and during sleep.

You will be required to return the accelerometer during final visit at the end of week 8.

Each study visit should take no longer than 2 hours. The research team will be available for support and contact throughout the course of the 9 weeks. Below is a schematic of laboratory visit 1 and 2 (Week 1 and week 8).



For participants in the exercise only and exercise and Mediterranean diet groups:

Exercise Intervention: Training Protocol

You will be required to participate in 3 x 20-minute sessions per week of unsupervised home-based HIIT exercise for 8 weeks, in which you will use the provided exercise protocol as guidance. The exercise protocol will gradually increase in intensity and will eventually involve 10 sets of repeated 1-minute bouts of high-intensity exercise, interspaced with 1-minute of jogging on the spot. During the high-intensity bouts of exercise, you will be advised to achieve ≥80% of your predicted heart rate maximum. This will be reflected as at least 17 out of 20 on the scale of on your Rate of Perceived Exertion (RPE). This is further explained on your exercise protocol booklet. You will be able to see this on the heart rate device. The 1-minute intervals will compose of bodyweight exercises with no rest. These exercises are detailed in your exercise protocol booklet, ranging from low, medium and high impact upon which you will choose to complete, based on which fits your needs best. Online HIIT video material will also be available online for access to demonstrate the exercise movements in a safe manner.

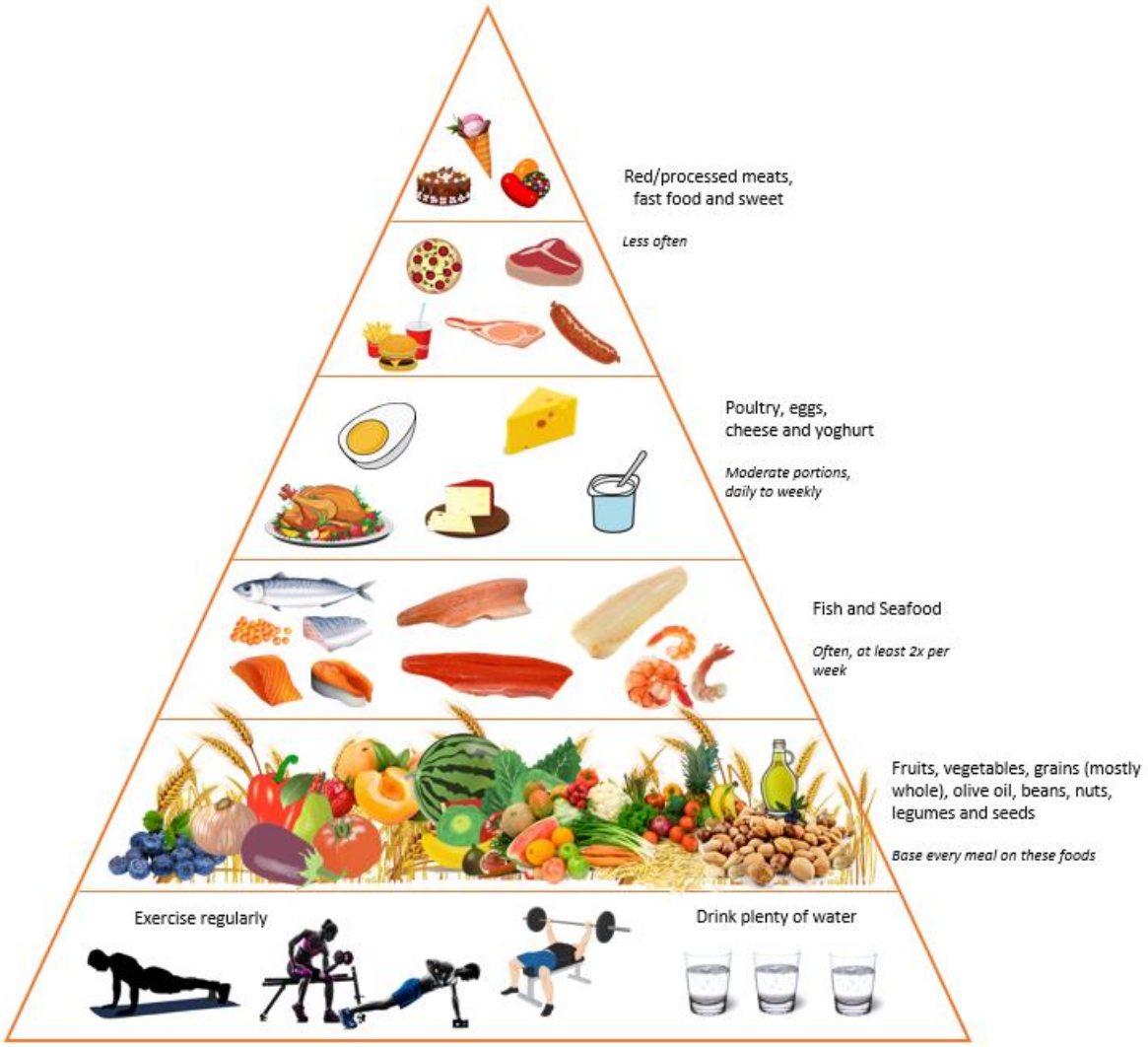
During week 1, you are advised to complete at least four sets of intervals. In week 2, the number of sets to be completed will increase to 6 sets. In week 3 and 4, the number of sets to be completed will be increased to 8. By week 5-8, you would be encouraged to complete 10 sets of intervals until the end of the exercise intervention. A schematic table depicting the 8-week exercise schedule will be available on your exercise protocol booklet. At the end of every exercise session, you would be asked to send information related to your perceived exertion (according to the RPE scale) and exercise completed to the researcher via an encrypted messenger service (Whatsapp).

You will also be invited every fortnightly (week 2, 4 and 6) to an in-person touch-base meeting with a member of the research team. This may involve a supervised training session to assess your ability and progress, and to answer any potential questions you may have. This may include exercising with the fitness instructor of your chosen exercise protocol. These sessions will strictly adhere and be dependent on the health and safety government guidelines at that time.

For participants in the exercise and Mediterranean diet group:

On top of exercise 3 x 20 minute sessions per week, you will be asked to adhere to a Mediterranean diet as discussed with the researcher during your laboratory visit 1. In brief, this follows a diet mainly based around plant-based foods, legumes and whole grains, moderate consumption of cheese, yoghurt, fish, poultry, and low consumption of red/processed meat and alcohol with a focus of utilising olive-oil as the main source of fat. This is summarised by a food pyramid below. The Mediterranean diet and its benefits through scientific evidence is further explain on your Mediterranean diet booklet. You will also be provided sheets of the Mediterranean Diet Score Tool to complete at the end of every week. This allows yourself and the researchers to assess your adherence to the diet throughout the 8-weeks.

During your fortnightly touch-base meeting with the researcher, this will involve discussion of your previous fortnight's progress with your diet in accordance to the completed Mediterranean Diet Score tool, as well as any questions and queries with regards to your progress in both the exercise and the diet.



For participants in the control group:

You will be asked to maintain your diet and physical activity levels throughout the 8 weeks. Your role as a control in this study is equally as important as the intervention groups, as you allow us to examine the full effects of the intervention. You will be contacted at the end of every week to aid compliancy and answer any potential questions or queries. In addition, a member of the research team will always be available to contact via the provided contact details below.

Will I get paid for participating?

There are no payments for taking part in this study.

What are the benefits of taking part?

The direct benefit from taking part in this study will be a metabolic health screening and improvements in health related to the study. Depending on your allocated group, you will be able to keep the study resources (such as the exercise protocol booklet, Mediterranean diet booklet, and access to the exercise videos) to promote a continued healthy lifestyle at the end of the study. Additionally, the information we get from this study might help to improve the

treatment of people who are overweight and at risk of developing metabolic and cardiovascular conditions.

What if something goes wrong?

Participating in the study will not put you at any increased risk of anything going wrong. Part 2 of this sheet provides information on how to deal with any problems if they arise.

Will my taking part in this study be kept confidential?

Yes, however you may be invited to join a group message thread (WhatsApp) and participate in group exercise sessions to maintain your morale over the 8-week period - this is 100% optional. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2

What will happen if I do not wish to carry on with the study?

Your participation in this study is completely voluntary and you may refuse to participate and are free to withdraw from the study at any time. If you withdraw from the study, we will dispose of the samples and withdraw them from the research.

What if there is a problem?

Participating in the study should not put you at any increased risk of anything going wrong. If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions or telephone Dr Rachel Churm (01792 604637). If you remain unhappy and wish to complain formally, you can do this through the following contacts.

For data issues –

If you wish to raise a complaint on how we have handled your personal data or if you want to find out more about how we use your information, the data controller for this project will be Swansea University. The University Data Protection Officer provides oversight of university activities involving the processing of personal data, and can be contacted at the Vice Chancellors Office: dataprotection@swansea.ac.uk. Your personal data will be processed for the purposes outlined in this information sheet.

For Health issues –

Then please contact the local health boards, Health watchdog. SBU Community Health Council

First Floor, Cimla Hospital, Neath, SA11 3SU

Website: http://www.wales.nhs.uk/sitesplus/902/home

For Management issues –

If there is a problem arisen with the management of the study then please contact the Head of College, Prof Huw Summers:

Professor Huw Summers

College of Engineering, Swansea University

[redacted]

[redacted]

What are the possible disadvantages and risks of taking part?

Some of the procedures in this study, such as the recording of your weight, height, full body DEXA scan and blood pressure present no risk to you. Other procedures, such as taking blood samples, can cause mild discomfort. The risks of taking a blood sample include: slight discomfort when the needle is inserted and possible bruising and a localised infection. These procedures will only be carried out by trained professionals that have received local training in phlebotomy and care will be taken to minimise pain and discomfort from the procedure.

For participants in the exercise groups: You will be exercising at home at your own risk. However during the exercise training, you will feel sensations associated with exercise, but these will constitute no greater risk than normal exercise activities.

For participants in the exercise and Mediterranean diet group: You may experience some discomfort when beginning a new diet since you will be adjusting to new foods and decreasing your intake of others. It is possible to experience light-headedness, bloating, gas and an upset stomach due to increase intake in fibre. Please make sure you alert your care provider of any prolonged discomfort you are experiencing related to dietary changes.

DEXA Radiation Risk

If you take part in this study you will have a whole-body scan by a DEXA machine. Some of these will be extra to those that you would have if you did not take part. These procedures use ionising radiation to form images of your body to provide our study with information on your body fat. Ionising radiation may cause cancer many years or decades after the exposure. We are all at risk of developing cancer during our lifetime. 50% of the population is likely to develop one of the many forms of cancer at some stage during our lifetime. Taking part in this study will add only a very small chance of this happening to you.

The amount of radiation used during a DEXA scan is less than 2 days' exposure to natural background radiation (NBR). By comparison, a flight to North America is equivalent to approximately a week's exposure to NBR, whilst a chest X-ray is equivalent to approximately 3 days' exposure to NBR.

How will my taking part in this study be kept confidential?

Any information and samples which are collected during this research will be kept strictly confidential. You will be given a unique identifying number and any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised. Some parts of your medical records and the data collected for the study may be looked at by authorised hospital staff to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

Involvement of the General Practitioner/Family doctor (GP)

It is not a requirement that your GP is informed of your participation in this study. However, consultation with your GP regarding participation of this study is of voluntary basis if you wish to.

What will happen to any samples I give and will genetic tests be done?

We consider the blood samples you donate for the research to be a gift. This sample will be used to look how exercise changes influences fat storage and stomach proteins. No genetic tests for disease risk will take place. Samples will be stored by the investigators in a locked freezer within a locked laboratory in Swansea University. The anonymised samples may be kept after the duration of this particular research study.

What will happen to the results of the research study?

The results of the study may be published in a medical journal and might be presented to other doctors and scientists with an interest in diabetes. If reports or publications are generated from the study you will not be identified in these. If you wish to receive general information on the results, you can contact one of the researchers via the email provided below. The results will also be used to look at the possibility of a larger more focused study involving the understanding on the role of exercise and its relationship with stomach proteins and fat storage.

Who is organising and funding the research?

This work is being organised between St David's Medical Foundation and College of Engineering Swansea University as part of a PhD.

Who has reviewed the study?

This study was reviewed by the Swansea University Research & Governance team. This study was given a favourable ethical opinion for conduct by the West Midlands – Solihull Research Ethics Committee (REC).

How will we use information about you?

We will need to use information from you for this research project. This information will include your name and contact details. People will use this information to do the research or to check your records to make sure that the research is being done properly. People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead.

We will keep all information about you safe and secure. Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

What are your choices about how your information is used?

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study.

Where can you find out more about how your information is used?

You can find out more about how we use your information at www.hra.nhs.uk/information-about-patients/, or a leaflet available from the researcher by contacting the email below.

Who can I contact for further information?

This Patient Information Sheet and the Informed Consent Form contain important facts which you should consider when deciding whether you are willing to take part in this study. If at any time you have any questions about the study, your rights as a research participant, a study related injury or side effects, you should contact the team involved in the study; upon which they will be available by email and telephone.

Name: Dr Rachel Churm
Telephone: [redacted]
Email: [redacted]

Name: Abbigail Tan
Telephone: [redacted]
Email: [redacted]

Swansea University
Prifysgol Abertawe

International Physical Assessment Questionnaire (IPAQ) for: Investigation of an exercise-induced alteration of the ghrelin axis and its implications in prediabetes

Name of Researcher: Dr Rachel Churm

Participant Identification Number for this trial:

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous and moderate activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

28. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No ***If answer is 'no', skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

29. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing upstairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

☐ No vigorous job-related physical activity ***Skip to question 4***

30. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ hours per day

_____ minutes per day

31. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last **7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ days per week

☐ No moderate job-related physical activity ***Skip to question 6***

32. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

33. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking ***Skip to PART 2: TRANSPORTATION***

34. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**

_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you travelled from place to place, including to places like work, stores, movies, and so on.

35. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle ***Skip to question 10***

36. How much time did you usually spend on one of those days **travelling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**

_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

37. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place ***Skip to question 12***

38. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**

_____ **minutes per day**

39. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

40. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**

_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

41. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shovelling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard ***Skip to question 16***

42. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

43. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard ***Skip to question 18***

44. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

45. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home ***Skip to PART 4: RECREATION, SPORT AND LEISURE TIME PHYSICAL ACTIVITY***

46. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

47. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time ***Skip to question 22***

48. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**

_____ **minutes per day**

49. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time ***Skip to question 24***

50. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

51. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time ***Skip to PART 5: TIME SPENT SITTING***

52. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

53. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day**

54. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**

_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

Swansea University
Prifysgol Abertawe

POST-MENOPAUSE QUESTIONNAIRE for: Evaluation of lifestyle modification on cardiovascular risk in overweight and obese postmenopausal women: a ghrelin mediated response

These questions relate to menopause. We define menopause as beginning after you have had no menstrual cycles for ONE YEAR. You have been given this questionnaire because you have indicated that you are post-menopausal.

Name of Researcher: Dr Rachel Churm

Participant Identification Number for this trial:

6. What was the approximate date of your last menstrual period? _____

7. What age did your menstrual cycles first become irregular? _____

8. Are you post-menopausal? (Answer YES, if your last menstrual period was over one year ago?)
   YES **NO**

9. If post-menopausal, what age did you consider yourself post-menopausal? *write N/A if not applicable _____

10. Are you currently taking hormone replacement therapy (HRT)?

( ) YES, I am currently on HRT

If yes, how long have you been taking HRT? _____

( ) YES, I have taken HRT but do not currently

If yes, how long ago was this? _____

( ) NO, I do not and have never taken HRT

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

Swansea University
Prifysgol Abertawe

GHR-exD

Home-based high intensity interval training study

Outline of the training program:

You should train 3 times per week. These sessions can be on any day of the week, and a day's rest between sessions. You can complete each session wherever you want.

Training protocol table (weekly):

You are advised to follow these timings for your exercise sessions. However, you can adjust the intensity accordingly to suit your fitness.

Training Type	**Week**															
	1		2		3		4		5		6		7		8	
High intensity	4x	60s	6x	60s	8x	60s	8x	60s	10x	60s	10x	60s	10x	60s	10x	60s
Low intensity (recovery)	4x	4min	6x	2min 30s	8x	1min 30s	8x	1min 30s	10x	60s	10x	60s	10x	60s	10x	60s
Total Time:	20 mins		21 mins		20 mins		20 mins		20 mins		20 mins		20 mins		20 mins	

Table 1. **Weekly Training Protocol Table**

During each high-intensity interval, you should aim to achieve a heart rate equal to/above your heart rate goal. This can be monitored using your heart rate monitoring device.

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

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

Warm up and cool down consists of 5 minutes of these exercises chosen by yourself:

- Neck stretch
- Shoulder stretch
- Tricep stretch
- Pelvic stretch
- Quad stretch
- Ankle-holding stretch
- Arm, leg and torso stretch
- Arm rotations
- Torso rotations
- Hip rotations
- Marching on the spot
- Jogging on the spot
- Lunges stretch
- Jumping jacks (or modified)

Low impact exercise:

- Burpees with no jump
- Side squat walk
- Plank shoulder taps
- Plank to opposite toe touch
- Jumping jacks (modified)

Moderate impact exercise:

- Mountain climbers
- Butt kicks
- Squat + kick
- Squat to toe touch
- Plank up/down
- Lunges (no jumping)

High impact exercise:

- Burpees
- Jumping jacks
- Lunge jumps
- Squat jumps
- Plank jumps
- Twisting mountain climbers
- High knee jogging

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Rate of Perceived Exertion (RPE) Scale

You should aim for **17 – 19 on the RPE scale.*

Rating	*Perceived Exertion*
6	*No exertion*
7	*Extremely light*
8	
9	*Very light*
10	
11	*Light*
12	
13	*Somewhat hard*
14	
15	*Hard*
16	
17	*Very hard*
18	
19	*Extremely hard*
20	*Maximal exertion*

Table 2. **Rate of Perceived Exertion Scale**