The role of metabolic rate and substrate utilization in the maintenance of body weight, body composition and insulin sensitivity

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Julia H Goedecke; Amy E Mendham; Louise D Clamp Pamela A. Nono Nankam[1], Melony C. Fortuin de Smidt[1], Lindokuhle Phiri[1], Lisa K. Micklesfield[1,3]; Dheshnie Keswell[1]; Nicholas J. Woudberg[4]; Sandrine Lecour[4]; Ali Alhamud[5], Mamadou Kaba[6], Faith M. Lutomia[6], Paul J. van Jaarsveld[2,7], Anniza de Villiers[2], Steven E. Kahn[8]; Elin Chorell[9], Jon Hauksson[10], Tommy Olsson[9]; **An exercise intervention to unravel the mechanisms underlying insulin resistance in a cohort of black South African women: Protocol for a randomized controlled trial**; *JMIR Research Protocols (2018)*, vol. 7(4) e75

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CONFERENCE PROCEEDINGS

Annual Meeting for the International Society for Behavioral Nutrition and Physical Activity (June 2016); Louise D Clamp, David J Hume, Tertius A Kohn, Estelle V Lambert, Jacolene Kroff, **Physiological and Metabolic Responses to a High-fat Meal Ingestion in Reduced Weight and Relapsed Weight Women: The Mind the Gap Study.**

Meeting of the Association for Dietetics in South Africa in the Western Cape (July 2016); Louise D Clamp, David John Hume, Estelle V Lambert, Jacolene Kroff*;* **Reduced insulin resistance in successful weight loss maintainers**

International Congress of Endocrinology (December 2018), Society for endocrinology metabolism and diabetes of South Africa; Louise D Clamp, Amy Mendham, Jacolene Kroff, Julia H Goedecke; **Higher fat oxidation in high VO_{2peak} responders promotes fat mobilization in response to exercise training**.

ABSTRACT

Obesity treatment requires approaches that target the reduction of body weight and fat mass. The improvement of cardiorespiratory fitness (CRF), metabolic flexibility and insulin sensitivity also contribute towards reducing obesity-associated risk factors. While energy restriction alone results in significant weight loss, exercise-only interventions provide small amounts of weight loss and prevent weight gain, while also improving many of the other variables targeted in obesity treatment. Once achieved however, successful weight-loss maintenance is challenging, with many individuals subsequently experiencing weight regain. The main objectives of this thesis were to explore the role of metabolic rate and substrate utilization in influencing body weight, body composition and insulin sensitivity. This two-part thesis hypothesised that: 1) exercise training, without dietary intervention, will improve metabolic rate and substrate utilization in a sedentary obese population, and that this would be associated with improved body composition, insulin sensitivity and CRF; and 2) metabolic rate, substrate utilization and insulin sensitivity are altered through weight loss/regain, predisposing these individuals to weight regain and impairing successful weight-loss maintenance.

In Part 1 of this thesis a 12 week exercise intervention in sedentary, obese (BMI 30-40kg.m^{-2}) black South African (SA) women (aged 22, IQR 21-24 years) was completed. Previous studies have shown that black SA women present with very low CRF, a key indicator of increased risk for non-communicable disease (NCD), and have a high prevalence of obesity and insulin resistance (IR). They are thus at increased risk for developing type 2 diabetes (T2DM). Furthermore, physiologically black SA women have also been shown to have less visceral adipose tissue (VAT) and more peripheral gluteal fat mass (FM) compared to their white counterparts, but are paradoxically more IR. Despite this presentation, to date there has been no supervised exercise intervention studies undertaken in this very high risk population group.

The first study of this thesis (chapter 2) aimed to assess the effects of the exercise intervention on changes in CRF, energy expenditure (EE) and substrate utilization, both at rest and during steady-state exercise compared to non-exercising controls. It also assessed baseline and changes in these measurements in relation to changes in body composition. Black SA women (BMI 30-40 kg.m^{-2}, 20-35 y) were recruited and randomized into control

(CTL, n=15), or exercise (EXE, n=20) groups. The CTL was instructed to maintain usual activity while the EXE completed 12 weeks of combined resistance and aerobic exercise training (4d.wk^{-1}, 40-60min.d^{-1} @ >70% peak heart rate (HR_{peak})). At pre-intervention, a treadmill-based CRF test, measuring peak volume of oxygen consumption (VO_{2peak}), was carried out. Thereafter resting and steady state exercise (50% VO_{2peak}) energy expenditure (EE) and respiratory exchange (RER) were measured along with body composition (dual-energy X-ray absorptiometry (DXA)). A frequently sampled intravenous glucose tolerance test (FSIGT) was also carried out to determine changes in insulin sensitivity. These tests were repeated at post-intervention testing with steady state testing being carried out both at the same relative intensity (50% post-testing VO_{2peak}) and the same workload (treadmill speed and gradient) as used for pre-testing. Dietary intake (4d diary) and daily step-count (ActivPAL) data was collected at pre-testing, 4, 8 & 12 weeks. Results showed that all participants had very low baseline CRF, falling below the 20th percentile previously shown in African American women. In response to exercise training, CRF increased by ~11% and rates of fat oxidation during steady-state exercise were improved, while in controls these remained unchanged. Compared to CTL, EXE also showed small but significant reductions, in weight, as well as BMI, waist (WC) and hip (HC) circumferences. In contrast weight, BMI and WC increased in non-exercising controls. Gynoid FM (absolute FM and as a proportion of total FM), rather than visceral adipose tissue (VAT), was reduced in exercise participants. Within the exercise group higher baseline fat oxidation rates during steady state exercise and lower resting carbohydrate oxidation rates explained 61.6% ($p<0.001$) of the variability in changes in gynoid FM in response to 12 weeks of exercise training in this group. In conclusion, exercise training improved CRF and fat oxidation rates during submaximal exercise in sedentary, obese black SA women. Higher fat oxidation rates during steady state exercise and lower resting carbohydrate oxidation rates at baseline were associated with the mobilization of gynoid FM in response to exercise training, rather than VAT as is typically shown in exercise interventions. This novel finding potentially represents an ethnic/gender specific response to exercise training. Further studies are needed to confirm this. Similar exercise training programs, that are sustainable over the long term, would therefore be beneficial in achieving meaningful increases in CRF while also supporting weight management and body composition improvements in this high risk population group.

Using data from the exercise intervention in obese black SA women, the second study of this thesis (chapter 3) investigated inter-individual variability in the CRF response (ΔVO_{2peak}) to

exercise training. The study specifically aimed to compare changes in EE and substrate utilization at rest and during steady state exercise, body weight and composition and insulin sensitivity between high and low CRF responders to the 12 week intervention. Furthermore it aimed to explore associations between baseline metabolic rate, EE and substrate utilization and subsequent changes in CRF in response to exercise training, to determine if baseline variability in these measures contributed to inter-individual variability in the CRF outcome. Within the exercise group, high inter-individual variability in CRF response to exercise training was identified. Based on a median split in ΔVO_{2peak}, high responders (HRS, n=10) increased CRF by 21.7 ±10.0% ($p<0.001$) compared to no change in both low responders (LRS, n=10; +0.6 ±6.3%, p=0.748) and CTL (-3.2 ±10.8%, p=0.195). This occurred despite all groups having similar baseline VO_{2peak} and the exercise group receiving the same exercise dose (number of exercise sessions attended and average intensity of the exercise sessions). At baseline, HRS derived ~62% of energy expenditure from fat oxidation during steady-state exercise compared to just 41% in LRS, who relied to a greater extent on carbohydrate oxidation. Furthermore, HRS were ~11 kg lighter than LRS. There was also a positive association between BMI and RER such that individuals with higher BMI showed lower fat utilization (i.e., higher RER). HRS reduced gynoid FM whereas in LRS this remained unchanged. This is in line with the findings of the previous chapter which showed that exercise-related reduction in gynoid FM was associated with greater baseline fat oxidation. LRS showed improvements in insulin sensitivity compared to CTL and HRS. Using regression analysis including the exercising participants, greater baseline carbohydrate oxidation rates both at rest and during steady state exercise predicted a poorer CRF to exercise training, explaining 37.5% of the variability in ΔVO_{2peak}. To the best of my knowledge, this is the first study to show that baseline variability in substrate utilization among sedentary obese individuals contributes towards explaining the variability in the CRF response to exercise training. However, further studies are required to confirm these results. Together, these studies show that higher fat oxidation rates are necessary for FM mobilization, while correspondingly reduced reliance on carbohydrate oxidation both at rest and during exercise supports improvements in CRF in response to exercise training. These findings add to a growing body of research aimed at explaining inter-individual variability in exercise intervention outcomes and may contribute to individualizing the exercise prescription.

Part 2 of this thesis used a cross-sectional approach and investigated firstly whether there was evidence for metabolic adaptation to weight loss/regain in response to long term weight maintenance, potentially predisposing individuals to future weight gain/regain. Secondly, I investigated whether insulin sensitivity is altered as a result of prior weight loss history, or whether successful weight loss restores insulin sensitivity to levels that are comparable to phenotypically similar controls with no weight loss history. Weight stable, BMI-matched South African women aged 20-45 years with or without a history of prior weight loss were screened and recruited. Four groups were defined as follows: Weight Reduced (RED, n=15) – lost at least 15% of body weight & maintained a reduced weight (BMI $\leq$ 27kg.m^{-2}) for over 12 months (<5% fluctuations); lean stable-weight (LSW, n=19) controls with a lean phenotype (BMI $\leq$ 27kg.m^{-2}) – no weight loss history; weight-loss relapse (REL, n=11) – lost weight (>15% of body weight), but relapsed back to overweight or obese (BMI $\leq$ 27kg.m^{-2}); and overweight or obese (BMI $\leq$ 27kg.m^{-2}) stable-weight controls (OSW, n=11) – no history of significant weight loss.

The first study in Part 2 (Chapter 4) compared metabolic rate and substrate utilization in RED and REL to their respective BMI-matched controls with no weight loss history, both at rest and in response to a high fat meal challenge. Metabolic rate and substrate utilization were measured both at rest, immediately after consumption of the high fat test-meal and every hour thereafter for three hours. Dietary intake (3 x 24h recalls) and physical activity (ACTi Graph GT3X accelerometer worn for 7 days) data was collected and body composition was measured (bioelectrical impedance, BIA). Questionnaires were also completed covering weight history, socio-economic status and eating behaviour. Results showed that there was no difference in either resting EE or substrate utilization between the RED and REL compared to the respective BMI-matched controls, after accounting for fat free mass (FFM). The TEF, postprandial EE (absolute and per kg FFM), post-prandial energy balance, RER, fat oxidation rate and post-prandial fat balance were similar between RED and REL compared to their respective controls, indicating that there was no evidence of metabolic adaption to weight loss. However, successful weight-loss maintainers did show behavioural strategies that may have counteracted weight-loss associated adaptive thermogenesis and supported weight-loss maintenance. These individuals had manipulated macronutrient intake (increasing protein and reducing carbohydrate intake), were more physically active, exhibiting less sedentary behaviour and increased moderate and vigorous activity, and had greater fat free soft tissue mass (FFSTM). While the presence of adaptive thermogenesis is not disputed in these results,

the distinct physiological and behavioural differences together observed in the RED may have been instrumental in attenuating weight-loss associated declines in EE, shown to persist into weight-loss maintenance. Together with these lifestyle strategies, weight reduced individuals also reported greater dietary restraint in comparison to controls. This is surprising after such a significant period of weight-loss maintenance (median weight-loss maintenance: 30 months) and highlights the ongoing challenges to maintain reduced weight. These findings contribute to the relatively smaller body of research into the longer-term persistence of weight-loss associated adaptive responses in comparison to that covering the acute weight loss phase. It also highlights strategies that may be effective in counteracting metabolic adaption to weight loss. As such, these strategies may warrant inclusion as part of weight-loss maintenance programs as they potentially help to reduce the risk for weight regain as a result of weight-loss associated adaptive thermogenesis.

The next study in Part 2 of the thesis (Chapter 5) aimed to examine the impact of successfully maintained weight loss and weight-loss relapse on insulin sensitivity compared to BMI-matched controls without a weight loss history. Predictors of variability in insulin sensitivity were also explored. Following the measurement of resting metabolic rate and substrate utilization a 75g oral glucose tolerance test was used to determine fasting and 2hr plasma glucose and insulin. The Homeostatic Model Assessment (HOMA-IR) and insulin sensitivity index ($ISI_{(0,120)}$) were used to assess insulin sensitivity. A novel finding of this study was that successfully maintained, weight-reduced individuals displayed enhanced measures of insulin sensitivity (lower HOMA-IR and higher $ISI_{(0,120)}$ measurements), compared to all other groups, including BMI-matched controls with no weight loss history. Previously studies have investigated changes in insulin sensitivity in response to weight loss and in weight-loss maintenance, but not necessarily in comparison to individuals without a weight loss history as defined by this study protocol. With weight regain however, insulin sensitivity measures for REL were not different compared to either LSW or OSW, showing that enhanced insulin sensitivity accompanying weight loss is likely reversed with weight regain. Prior weight history, fasting substrate utilization, measures of body weight and composition, protein intake per kilogram, physical activity and CRF were all associated with measures of insulin sensitivity. Using these variables in regression models, ~60% of the variability in insulin sensitivity in both HOMA-IR and $ISI_{(0,120)}$. Weight loss and weight regain history followed by fasting RER were the most significant independent predictors of insulin sensitivity. In conclusion, a novel finding was that successfully weight-reduced individuals are more insulin

sensitive than their BMI-matched controls with no weight loss history, independent of dietary intake and physical activity. This remains evident even after significant periods of maintaining the reduced weight. Weight loss maintenance programs are essential to retaining metabolic benefits acquired through weight loss. Remaining physically active by reducing sedentary behaviour and in particular including small amounts of vigorous physical activity significantly predicts improved insulin sensitivity.

This thesis includes a number of novel findings. In Part 1, we showed that in response to exercise training gynoid FM, rather than VAT, was reduced in sedentary obese black SA women undergoing a 12 week exercise intervention, which may represent an important ethnic/gender specific response. We also showed that substrate utilization plays an important role in altering body composition and CRF in response to an exercise intervention. Greater fat oxidative capacity at the outset resulted in an enhanced ability to reduce gynoid FM in response to exercise training. Furthermore, a greater reliance on carbohydrate rather than fat oxidation during baseline testing predicted a poorer CRF response. Identification of individuals with a lower capacity for fat oxidation at the outset of an exercise intervention may therefore allow for a more targeted exercise prescription, which may in turn improve outcomes of exercise interventions. The lack of clinically significant weight loss suggests that future exercise interventions should prescribe exercise EE of sufficient magnitude to achieve weight loss and emphasize adherence to this prescription or include some dietary restriction. Education around the possible adaptive responses to increased EE and the imposed energy deficit, highlighting the strategies employed by weight reduced individuals from Part 2 of this thesis, may help to attenuate potential metabolic adaption to increased EE and further improve the weight loss outcomes of exercise-only interventions. It may also help to inform weight-loss maintenance programs to assist individuals to maintain the reduced weight following weight loss. The enhanced insulin sensitivity in weight reduced individuals as shown in Part 2, may potentially represent an ongoing and persistent adaptive response to weight loss that may in itself increase the risk for weight-loss relapse. Education around the physiological adaption to significant weight loss and emphasizing strategies that may counteract this metabolic adaptation may improve the efficacy of both weight-loss and weight-loss maintenance programs.

LIST OF ABBREVIATIONS

AEE	Activity associated energy expenditure
ASA24	Automated self-administered 24 hour diet recall
AUC	Area under the curve
BMI	Body mass index
BW	Body weight
CRF	Cardiorespiratory fitness
CTL	Control group
CHO	Carbohydrate
DXA	Dual energy x-ray absorptiometry
EB	Energy balance
EE	Energy expenditure
EI	Energy intake
EXE	Exercise group
FFA	Free fatty acid
FFM	Fat free mass
FFSTM	Fat free soft tissue mass
FM	Fat mass
FSIGT	Frequently sampled intravenous glucose tolerance test
h	hours
HC	Hip circumference
HOMA-IR	Homeostatic model assessment of insulin resistance
HRS	High VO_{2peak} responders
HR	Heart rate
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IMCL	Intramyocellular lipid content
IR	Insulin resistance
$ISI_{(0,120)}$	Insulin sensitivity index
kcal	Kilocalories
kJ	Kilojoule
kg	Kilogram
L	Litres

LRS	Low VO_{2peak} responders
LSW	Low weight stable weight control group with no weight loss history
NCD	Non-communicable disease
NREE	Non-resting energy expenditure
OGTT	Oral glucose tolerance test
OSW	Overweight/obese stable weight control group with no weight loss history
PAL	Physical activity levels
PFC	prospective food consumption
RED	Weight reduced, successfully maintained
REL	Weight loss relapsed individuals
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
SAT	Subcutaneous adipose tissue
S_I	Insulin sensitivity measurement determined from the FSIGT using Bergman's minimal model of glucose kinetics
TDEE	Total daily energy expenditure
TEF	Thermic effect of feeding
TFEQ	Three factor eating questionnaire
T2DM	Type two diabetes mellitus
VAS	Visual analogue scale
VAT	Visceral adipose tissue
VO_{2peak}	Peak volume of oxygen consumption
VO_{2max}	Maximal volume of oxygen consumption
WC	Waist circumference
WHR	Waist-to-hip ratio

CHAPTER 1

1. Introduction and Literature Review

1.1 Introduction

The prevalence of obesity has increased globally, from 105 million adults in 1974 to 640 million in 2014[1]. With increasing body weight there is increased risk of non-communicable diseases (NCD) such as type 2 diabetes (T2DM), cardiovascular disease and certain cancers[2–5]. Likewise, mortality risk is increased almost twofold with body mass index (BMI) greater than 35 $kg.m^{-2}$ compared to normal weight (BMI: 18.5-24.9 $kg.m^{-2}$) individuals[5]. Furthermore, a history of prior overweight and obesity increases mortality risk in normal weight individuals compared to those who had never exceeded normal weight[5]. This link between obesity and NCD means that with increasing prevalence of obesity, a growing burden is placed on healthcare services[6,7]. Estimates suggest that obesity accounts for 31.8% of health care costs and around 68.1% of indirect costs attributed to reduced productivity[8]. Computer models combining UK and US populations have predicted that if obesity levels continue to rise, by 2030 there will be an additional 6-8.5 million cases of diabetes, 5.7-7.3 million cases of cardiovascular disease, 492,000-669,000 cancer cases and the loss of 26-55 million quality-adjusted life years. The *additional* medical costs associated with treating these preventable diseases of lifestyle are estimated at U$48-66 billion for USA and £1.9-2 billion for the UK[9]. For the UK alone it is predicted that by 2050, 60% of men and 50% of women will be obese and that obesity-related illness will cost the British healthcare service around £45.5 billion a year[10].

While obesity was previously most prevalent among affluent societies, it has shifted to more "population-wide obesity", including poorer segments of society. It is also rapidly increasing in developing countries among children and adults, where the rural-to-urban transition has increased the availability of cheaper, nutrient poor, calorie rich foods, causing a shift away from traditional higher fibre ethnic diets[11]. This is coupled with the built environment which is less conducive to physical activity[1,11,12], including the reduced 'walkability' of neighbourhoods and safety from traffic and crime[13,14]. In southern Africa, estimates from 1980 to 2014 show that the absolute age-standardized BMI, particularly among women, not only exceeded global averages but was also increasing at a faster rate[15]. This data also continues to show a strong correlation between BMI and diabetes prevalence, particularly amongst southern African women[15,16]. Within the southern African region, South Africa (SA) has the highest age-standardized mean BMI, having increased substantially from 1980 to 2014 (men from 22.8 to 24.8 $kg.m^2$; women from 26.7 to 29.2 $kg.m^2$), and the highest

prevalence of T2DM (men 9.7%; women 12.6%)[17]. In accordance with this increase in mean BMI, currently 68% of women and 31% of men in SA are classified as overweight or obese and this is accompanied by increasing hypertersion[18]. Obesity rates are also increasing rapidly among adolescent girls, attributable largely to nutritional deprivation in early childhood, higher adult socio-economic status and cultural perceptions of larger body weight being considered normal[19]. In SA, age-standardized mortality rates associated with NCD, of which cardio-vascular disease is a leading contributor, now exceed that of HIV/AID and tuberculosis combined[18]. Furthermore, among urban dwelling black South Africans, age-standardized T2DM prevalence has increased by ~53% from 1990 to 2008/9 (men 11.3%; women 14.7%), exceeding the national average, with greater prevalence of cardio-vascular risk factors observed amongst these individuals[20].

Low physical activity levels (PAL) are directly associated with the development of obesity and together with low cardio-respiratory fitness (CRF), are independently associated with increased risk for chronic disease and mortality[21–23]. Exercise training is a type of physical activity that specifically aims to enhance elements of physical fitness such as CRF, which describes the capacity of the cardiovascular system to supply oxygen to the working muscles in order to sustain physical activity. PAL is a major determinant of CRF, besides age, gender, sex, health status and genetics, and as such CRF is often used as an indicator of recent PAL[24]. However, PAL and CRF have different associations with NCD risk. While increasing PAL has been shown to result in a linear decrease in NCD risk, CRF at or below the 20th percentile is associated with significantly greater health risks, which decline dramatically with increasing percentiles of CRF[25,26]. CRF is typically measured as the maximal or peak volume of oxygen consumption (VO_{2peak}) during an exercise test to exhaustion[21] and is a key aspect of physical fitness that is strongly associated with the prevention of NCD[24,27]. The inclusion of CRF with traditional NCD risk factors (e.g. Smoking, hypertension, hypercholesterolemia and T2DM) substantially improves the prediction of NCD risk[27]. Indeed unfit normal-weight individuals have been shown to have double the mortality risk of fit counterparts, while obese and normal-weight fit individuals had similar mortality risks[28]. Furthermore, increasing CRF among obese individuals is associated with a reduction in all-cause mortality in comparison to individuals where CRF declined[22]. In contrast, although T2DM risk reduces with increased fitness among obese individuals, it remains higher in obese unfit compared to normal-weight unfit individuals[29]. Thus while increased CRF is emphasized in the reduction of NCD risk even without weight loss[28,30], it does not completely eliminate obesity-associated health risk

and therefore weight loss together with reduced adiposity remain important elements of obesity treatment[26,31]. Even so, improving CRF is of significant importance in reducing NCD risk, particularly given the disproportionately higher health risks among individuals falling in the lowest 20th percentile of CRF.

Strategies are required to address the increased health risks associated with the obesity epidemic. This literature review (see Figure 1.1 below) is not intended as a comprehensive review of all aspects of obesity development, but rather is focused specifically on highlighting gaps in the literature and introducing the aspects of obesity development and treatment that will be addressed in my research questions. The review initially focuses on understanding the aetiology of obesity, specifically addressing the role of energy expenditure (EE) and substrate utilization in maintaining energy and macronutrient balance. Hereafter the review focuses on the factors linked to obesity-associated insulin resistance (IR) and metabolic inflexibility, together with the involvement of altered fat oxidative capacity. In the treatment of obesity, weight loss together with improvement of CRF, fat oxidative capacity, body composition and insulin sensitivity are important targets. The following section then reviews weight loss results achieved through dietary interventions compared to exercise training interventions, and then goes on to review the additional benefits of exercise training in targeting other aspects of obesity treatment mentioned above. Once achieved, successful weight loss maintenance becomes critical in order to prevent weight regain. The evidence for physiological changes accompanying weight loss, that increase the risks for weight-loss relapse, both in the acute weight-loss phase as well as in longer term weight-loss maintenance, are then considered.

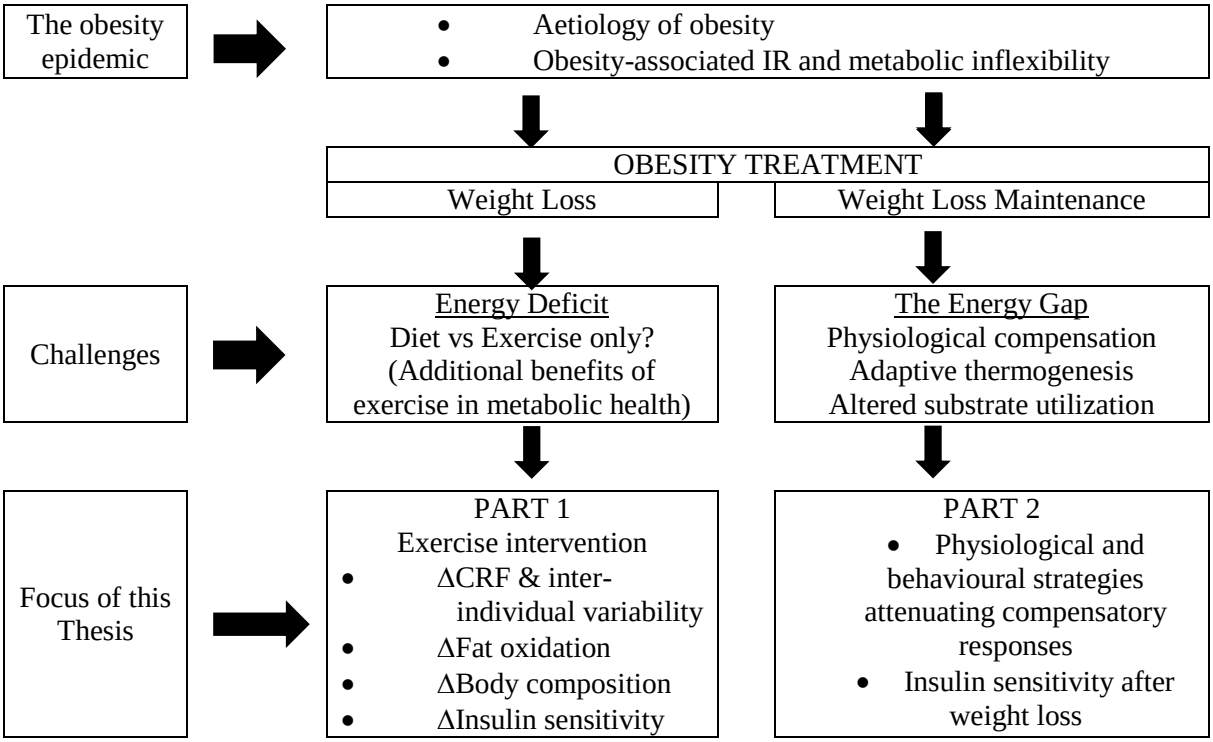

Figure 1.1: Structure of the literature review.

Note: IR: insulin resistance; CRF: cardiorespiratory fitness

1.2 Aetiology of obesity

Obesity, a condition defined by increased body fat mass, is largely attributed to the overconsumption of calorie dense foods coupled with high levels of sedentary behaviour and low levels of physical activity[4,32]. However energy balance is complex and influenced by a multifaceted interplay between environmental, behavioural, physiological and genetic factors[4]. Mood disorders, side effects of medications and food addiction are also increasingly recognised in the aetiology of obesity[4]. Among environmental factors, oversupply of inexpensive, highly palatable, energy dense but nutrient poor food options, advertising policy and cultural and social norms are highlighted as influencing increased energy intake (EI), while the built environment, screen time, cost and access to exercise facilities and clubs as well as school policy on exercise are implicated in reduced opportunities for voluntary energy expenditure (EE) and PAL[33]. Behavioural factors include increased EI and eating patterns, sedentary lifestyle, reduced PAL, inadequate sleep and perceived lack of available time[33,34]. Physiologically energy homeostasis in itself is highly complex, involving the central nervous system integration of both external and internal inputs in order to orchestrate changes to EI and EE, restore energy balance and maintain body weight stability. However, malfunctions

within this intricate system may predispose individuals to obesity development and this is an vast area of ongoing investigation[35]. Environmental influences, coupled with emotional and reward aspects of food intake can also result in these homeostatic mechanisms being consciously overridden[35–37]. While acknowledging these factors in the aetiology of obesity, this review and thesis focuses specifically on alterations in components of EE and substrate utilization implicated in obesity development through their influence on body weight, body composition and insulin sensitivity.

1.2.1 Energy expenditure and energy balance

Lower total daily EE (TDEE) is implicated in facilitating a positive energy balance (EI > TDEE) that underlies obesity development and can undermine weight loss and weight-loss maintenance. TDEE is made up of resting metabolic rate (RMR; ~55-75% of TDEE), the thermic effect of feeding (TEF; ~7-15%) and the balance by activity related energy expenditure (AEE), including both planned and spontaneous physical activity. These can be further divided into AEE intensities, i.e. minutes per day in sedentary, light, moderate and vigorous activity[38]. Fat free mass (FFM, which encompasses the organs, skeletal muscle and bone) drives RMR, with muscle mass and liver shown to explain 81% of the variance in RMR[39]. An inherently lower RMR is suggested to predispose certain individuals towards weight gain and obesity and predicts a reduced response to weight loss interventions[40,41]. Differences in FFM and its composition may also explain ethnic differences in RMR and its contribution to relative obesity prevalence, as shown in African American compared to white counterparts[40,42]. In contrast, adipose tissue is the least metabolically active component of body composition, contributing just 15-20% of the equivalent amount of FFM to RMR, which highlights the importance of maintaining FFM in order to prevent declines in RMR[43]. Nonetheless, greater adipose tissue mass in obesity leads to greater FFM[39], and therefore explains the higher absolute RMR observed in obese compared to lean individuals[35,43]. Besides body weight and composition, RMR is also influenced by other non-modifiable factors such as age and gender, as well as sympathetic nervous system activity and hormone action (leptin, thyroid hormones, insulin)[38]. The TEF refers to the increase in RMR associated with digestion, absorption and storage of nutrients consumed and can be influenced by the macronutrient composition of food intake. Fat intake increases RMR by 2-3%, carbohydrate intake by 5-8% while protein has a more pronounced effect on metabolic rate, increasing RMR by around 20-30%[44]. As such, dietary macronutrient composition can also influence

this component of TDEE. When adjusted for body weight and composition, obese individuals are shown to accumulate lower relative AEE, particularly moderate and vigorous activity, compared to lean counterparts[45], and this may be a significant contributor to ongoing weight gain in obesity. AEE is also highly variable, ranging between 15% of TDEE in sedentary individuals up to 50% in highly active individuals, thus activity patterns can significantly impact on TDEE[41]. Therefore, while a lower RMR contributes to reduced TDEE and a positive energy balance, low levels of physical activity may play a far more important role on the EE side of the equation in obesity development and offers a modifiable component in the treatment of obesity.

Obesity pathogenesis has been attributed to a two-fold process of 1) sustained positive energy balance and 2) a 'resetting of the body's "set-point" at an increased value'[35]. Over the short term, overfeeding studies show that both lean and obese individuals will similarly increase components of TDEE and reduce EI in order to restore energy balance and resist weight gain[46], and this is attributed to homeostatic neuroendocrine responses to the positive energy balance[35,47]. However over the longer term, for obesity to occur, ongoing increases in TDEE required to eliminate a persistent positive energy balance and resist weight gain, are necessarily weaker and not sustained in comparison to those resisting weight loss[35]. (See 'Weight loss maintenance' below for further discussion of adaptive responses to reduced weight). Although the basis for the defence of a higher, rather than lower, body weight set point is not fully understood, this is suggested to be the result of evolutionary survival advantage, selectively benefitting individuals able to increase body fat stores[48]. However, as alluded to in the previous paragraph, in the presence of powerful environmental and behavioural influences this would result in relatively unopposed weight gain in preparation for a threat that never emerges. Indeed, longitudinal studies show that even small positive energy balances, as low as 10 kcal (40 kJ) per day, if consistently applied, can result in average weight gain of 0.5 kg per year[49]. Nevertheless, rather than being a passive process whereby weight changes in response to changes in energy balance, evidence suggests that obesity development involves 'biological defence' of a higher body weight (and body-fat mass) set-point that more actively resists weight reduction compared to weight gain[35]. The development and maintenance of obesity is therefore increasingly shown to be the result of dysregulated energy homeostasis, weighted towards the defence of energy stores rather than protecting against obesity, and a better understanding of these mechanisms, and how to counteract them, is needed in obesity treatment[35].

While studies show average increases in body weight across various age categories over time, a large proportion of these individuals are able to maintain stable body weight[50]. This suggests a consistent ability to match EI and TDEE in order to achieve energy balance[35]. However, with an increasingly sedentary lifestyle, there appears to be increased dysregulation in the ability to match EI with TDEE, thus leading to overconsumption, weight gain and obesity development over time[35]. A seminal study in West Bengali mill workers demonstrated that at moderate to high levels of physical activity, termed the 'normal-activity zone', EI increased linearly with EE and resulted in stable body weights over a wide range of PAL. In contrast, sedentary office workers showed a decoupling of this association whereby EI increased, rather than decreased, with progressively lower PAL, and consequently resulted in increased body weight[51]. Thus a 'j-shaped' curve is described along a PAL continuum. The authors argued that sedentary behaviour is a recent phenomenon, considered to be "abnormal", potentially explaining why homeostatic mechanisms failed to respond to low PAL and reduced TDEE by appropriately decreasing EI accordingly[51]. The presence of this 'j-shaped' curve of EI against graded PAL is further supported by more recent studies which indicated improved ability to match EI with EE as regular exercise was adopted[52,53]. Therefore reducing sedentary behaviour and increasing PAL may improve the ability to more consistently match EI with EE and is an important consideration in obesity prevention and treatment[54].

1.2.2 Macronutrient Balance

In addition to energy balance, maintaining stable body weight over time also depends on maintaining stable body energy stores (i.e. glycogen and fat). This necessitates macronutrient balance; the ability to match carbohydrate and fat intake with their respective oxidation for energy production[55]. While the human body has unlimited capacity for fat storage, it is only able to store around 500-600g of carbohydrate in the form of glycogen (~500g in muscle and ~100g in the liver)[56]. As such, carbohydrate has oxidative priority over fat where storage capacity is unlimited and this is facilitated through the suppression of lipolysis and fat oxidation in response to insulin following acute ingestion of carbohydrates[55,57]. Therefore, over the short term, balance between carbohydrate intake and oxidation is generally achieved[58]. However if carbohydrate or fat intake appreciably exceeds oxidation, the balance must be accumulated in the body as glycogen or fat, respectively. In order to maintain stable body stores and body composition, corrective adjustments would then be required to restore

macronutrient balance[55]. Therefore the capacity for fat and carbohydrate oxidation is an important consideration for energy storage or utilization and thus for energy partitioning.

Flatt (1987) described a "two compartment model" which considered the relative intake of fat and carbohydrate in relation to their oxidation or storage. He proposed that when fat intake is increased, fat oxidation must also be increased in order to maintain macronutrient balance and prevent fat storage. This might be achieved through decreasing carbohydrate intake and thus reducing glycogen saturation of muscle in order to facilitate an increase in fat oxidation, as is shown in individuals habitually consuming a low carbohydrate, high fat diet[58,59]. If glycogen saturation remains unchanged while fat intake is increased, alterations to the fuel mix for energy production would be unlikely and the additional fat will thus be stored. Interestingly, in support of this model, negative carbohydrate balance (oxidation > intake, net glycogen utilization) and higher reliance on carbohydrate oxidation over 24 hours is shown to result in increased food intake over the following 3 days, potentially driven by the need to replace glycogen stores[60]. Similarly, lower RER (higher fat oxidation) during exercise (90 minutes at 60% VO_{2peak}) is also shown to be associated with reduced ad libitum food intake and a greater potential to be in negative post-exercise energy balance over the following 48 hours, compared to individuals with higher exercise RER[61]. The defence of glycogen stores thus suggests a mechanism by which weight gain and body composition changes may be influenced by substrate utilization. Another study in overweight and obese individuals on a standardized high carbohydrate diet (55% of TDEI) who were in positive carbohydrate balance (oxidation < intake, expansion of glycogen stores) over the 15 day test period, showed lower gains in body weight, fat mass and body fat percentage over the following 4 years compared to individuals who were in negative carbohydrate balance (oxidation > intake, net glycogen utilization), independent of S_I[62]. Several studies have also shown that a higher fasting respiratory exchange ratio (RER), indicating a greater preference for carbohydrate oxidation, predicts future weight gain[63–65]. Furthermore, a high non-sleeping RER predicted subsequent 2 year gain in fat mass and was shown to occur independently of energy balance, S_I and insulin secretion[66]. Evidence suggests that reduced fat oxidation, and a preference for carbohydrate oxidation and glycogen utilization, may increase risk for weight and fat mass gains as a result of both greater fat storage as well as increased EI potentially driven by the defence of glycogen stores[66].

1.3 Obesity-associated insulin resistance and metabolic inflexibility

With increasing obesity and adiposity there are concomitant increases in insulin resistance (IR) that are associated with the development of T2DM, cardiovascular disease, cancer and increased mortality[2–4]. Increasing BMI is associated with greater ectopic fat deposition (liver, pancreas, skeletal muscle)[67] and increased inflammation[68]. This precipitates lipotoxicity and impaired glucose metabolism which is associated with the degree of IR and mitochondrial dysfunction[69–72]. Related to this, obesity is also associated with increasing 'metabolic inflexibility', a reduced ability to match fuel oxidation to substrate availability[73]. This section reviews the literature to provide an understanding of the relationship between obesity and the development of IR and metabolic inflexibility. In the subsequent section, I review in more detail how these obesity-associated outcomes might be beneficially modified through exercise training.

1.3.1 Insulin resistance

Obesity and increased fat mass are associated with reduced insulin sensitivity and the increased risk for cardiovascular disease, hypertension and T2DM[74,75]. IR describes the reduced sensitivity of various tissues; of skeletal muscle to take up and utilize available blood glucose, and/or liver to inhibit hepatic glucose production and/or adipose tissue to inhibit lipolysis and release of free fatty acids into the circulation[57]. Pancreatic beta-cell insulin secretion must therefore be increased in order to normalize blood glucose levels, resulting in hyperinsulinemia that is causally associated with the development of obesity-associated T2DM[76]. Evidence increasingly suggests that while IR is associated with greater fat mass, certain regional body fat depots may play a disproportionate role in IR[74,75]. Earlier studies found that IR and its metabolic sequelae were significantly associated with visceral adipose tissue (VAT), despite its relatively smaller volume in comparison to abdominal subcutaneous adipose tissue (SAT)[77–80]. However, this view has since been challenged by a number of studies that have found similar associations between IR and both abdominal SAT and VAT[74,75]. Nonetheless, Goodpaster (1999) showed that while insulin sensitivity was associated with general and regional adiposity both before and after weight loss, the magnitude of improvement in insulin sensitivity was only correlated with percentage change in VAT, but not abdominal SAT[81]. Similarly, in a 7 year follow-up study Lemineux (1996) showed that a subgroup of women with the greatest increase in VAT showed the greatest

decline in glucose homeostasis, even after controlling for increases in body FM[80]. Therefore reduction of VAT remains an important target in the treatment of obesity and IR.

Metabolic dysregulation related to IR is argued to be driven by over-nutrition, rather than increased adiposity per se[76], and essentially represents an inability 'to safely partition a chronic fuel surplus'[82]. In line with this argument, the relationship between SAT and IR has been postulated to reside in its larger mass compared to VAT, and as such in its ability to absorb increased fat accumulation[74,83]. However, with increasing FM, diminished adipogenesis leads to subcutaneous adipocyte hypertrophy and together with reduced angiogenesis results in adipose tissue hypoxia, promoting an inflammatory profile associated with IR[74]. With adipocyte hypertrophy uptake and metabolism of glucose is also reduced, leading to increasing adipose tissue dysfunction involving lipid biosynthesis, endocrine and secretory functions[83]. Adipocyte hypertrophy ultimately signals that SAT is reaching capacity and with further positive energy balance is eventually unable to absorb additional fat accumulation[83]. This is hypothesized to bring about a 'tipping point' precipitating ectopic fat deposition in tissues unaccustomed to deal with excess fat storage (e.g., skeletal muscle, liver, pancreas), further increasing IR and hyperinsulinemia, eventually leading to T2DM[74]. The hypothesized 'tipping point' may differ between individuals based on gender, ethnicity or genetics and may potentially explain the metabolically healthy obese paradox[84]. This 'tipping point' can be indirectly determined from ectopic fat accumulation or SAT cell size relative to the ratio of adiposity-to-BMI and can occur in non-obese individuals[83]. Certainly within a SA context differences in fat distribution have been shown between black and white women[85] with black women presenting with lower VAT and more peripheral gluteal FM previously thought to be protective[86]. However, gluteal fat depots in black SA women also show differential gene expression compared to white women, pointing to greater risk for adipocyte hypertrophy[87,88], reduced vascularization[88,89] and increased hypoxia and inflammation[90], which was in turn associated with increased IR compared to white BMI-matched counterparts.

Exercise training consistently shows reductions in ectopic fat deposition in the liver, but not in muscle and this is not necessarily dependent on clinically significant weight loss[91–94]. Aerobic, but not resistance, exercise training was effective in inducing this response[91] but was not influenced by differences in either volume or intensity of the exercise stimulus[92] and may therefore be more dependent on overall EE. In overweight individuals, comparisons of 6

months of either calorie restriction (CR, -12.5%), increased exercise EE (CREX +12.5%) and low calorie diets (LCD, -15%) showed reductions in body weight (10-14%), adipocyte size and ectopic liver fat for all three intervention groups, but significantly improved insulin sensitivity only in LCD and CREX groups, but not CR ($p=0.08$)[94]. These studies suggested that exercise-induced increases in fat oxidation, particularly during and after exercise, increases plasma lipid clearance, thus accelerating the removal of hepatic lipid content. As alluded to above, there exists a small proportion of obese individuals are found to be metabolically healthy despite excessive adiposity[84,95]. Increased physical activity[96], greater CRF[31] as well as improved fat oxidative capacity[97] differentiate between metabolically healthy and unhealthy obese. In summary, IR accompanies obesity and involves dysregulation in energy partitioning and storage. Obesity treatment should therefore consider interventions that aim to reduce body weight and ectopic fat accumulation including calorie restriction, and physical activity.

1.3.2 Metabolic inflexibility

"Metabolic inflexibility" is described as a reduced capacity for fasting fat oxidation and an impaired ability to transition to carbohydrate metabolism under both insulin- and sympathetic-stimulated (e.g., during exercise) conditions, and is shown more often in obese compared to lean healthy individuals[73,98,99]. Metabolic inflexibility necessarily involves impaired insulin sensitivity of tissues, together with defects in fatty acid metabolism[100]. Skeletal muscle plays an important role in whole body fat oxidation, and studies in obese compared to normal-weight individuals have shown increased fatty acid transporters on skeletal muscle cell membranes, but reduced fat oxidative capacity and mitochondrial content, as well as lower concentrations of key proteins involved in mitochondrial fatty acid oxidation[101,102]. Therefore, in obese individuals, skeletal muscle appears to be primed to take up and preferentially store rather than oxidize fat, a feature that may persist even after weight loss[73,101]. However, metabolic flexibility is both associated with general, habitual PAL and may be modified by changes in PAL[103]. Bergouignan (2013) showed that individuals demonstrated a greater capacity to alter the postprandial fuel mix (increased variability in RER) in response to a smaller change in plasma insulin following exercise training[104]. This effect was reduced with detraining and sedentary behaviour[104]. Exercise training is also effective in increasing fat oxidation at rest and during exercise and can improve insulin sensitivity, thus promoting improved metabolic flexibility[100]. Hence metabolic inflexibility is

prevalent with obesity and more sedentary behaviour and may be beneficially altered through exercise training.

1.4 Weight loss strategies

Prevention of weight gain is a primary goal in reducing obesity prevalence and progression, while in obesity treatment, even small amounts of weight loss can have significant health benefits[105]. Computer simulations have shown that a 1% reduction in the predicted BMI in USA in 2020 could prevent 2.1-2.4 million cases of T2DM, 1.4-1.7 million cardio-vascular disease cases and gain ~16 million quality-adjusted life years[9]. Furthermore, following weight loss through diet and exercise, there is evidence that even with some weight regain, many metabolic benefits remain in place such as reduced systemic inflammation and improved insulin sensitivity[106]. In this section the effectiveness of diet compared exercise interventions are compared reviewing their effectiveness in achieving weight loss and improving metabolic risk factors. Thereafter evidence for the benefits of exercise in relation to improvements in CRF, fat oxidative capacity, body composition and insulin sensitivity are investigated.

1.4.1 Weight loss through energy deficit: diet versus exercise

To achieve weight loss a negative energy balance (EI < EE) is required and this can be achieved either through increased exercise EE, through dietary calorie restriction or a combination of both. Systematic reviews and meta-analyses over the years have generally agreed that the magnitude of weight loss from weight-loss interventions employing diet-only strategies (~11 kg after 12-16 weeks) is greater compared to interventions using exercise alone (~0-3 kg)[3,105,107]. Typically dietary interventions will impose an energy deficit of around 500-1000 kcal per day, whereas exercise prescription in many intervention studies is more in line with recommendations to improve metabolic health (150-250 minutes of moderate intensity exercise per week - equivalent to 1,200 to 2,000 $kcal.wk^{-1}$)[105,108]. More recent recommendations suggest that increased physical activity (> 250 minutes per week) is required to achieve clinically significant weight loss[105,107,108]. Even at this increased exercise training load, the energy deficit imposed through exercise is only ~300 kcal per day, significantly below that imposed in dietary approaches. Indeed, where the energy deficit imposed through diet- or exercise-only interventions are similar, similar weight loss is

shown[94,109,110]. Achieving the required energy deficit for weight loss also requires high levels of compliance to the exercise prescription, with better weight loss results shown in exercise interventions when adherence rates are very high[111,112]. However, compliance with very high workloads, particularly among sedentary obese populations, may prove challenging. Hence a combination of moderate calorie restriction together with exercise training is potentially a better option for weight loss interventions. Body composition changes should also be considered when assessing weight loss in response to exercise versus diet only interventions. Exercise training is shown to result in reductions in FM and increases in FFM, which would dilute body weight changes in response to the exercise intervention[107].

Without dietary restriction, compensatory responses to the energy deficit created through exercise are shown[113]. This is less evident in interventions of shorter duration and among younger individuals with higher initial FM[113]. Weight loss achieved through exercise-only strategies is also highly variable, with some individuals being more susceptible to compensatory behaviours (reduced EE and increased EI) that offset the exercise-induced energy deficit[114]. Compensatory responses to exercise interventions are generally shown to be related more to increased EI than reduced non-exercise related PA[111,115,116]. Certainly exercise intervention studies report changes in appetite hormones that would induce increased appetite (ghrelin), but at the same time show increase satiety (PYY, CCK, GLP-1) following food intake, both acutely[117,118] and over several weeks[119]. This exercise-induced increase in satiety-related hormones may also be associated with higher levels of exercise intensity[120]. However, it should also be noted that cross-sectional studies have shown 'constrained total energy expenditure' such that TDEE is more strongly correlated over lower physical activity ranges, increasing in a linear fashion with increasing PAL[121], but this relationship becomes weaker and plateaus over higher physical activity ranges. This indicates the adaptation of non-exercise related EE to longer term physical activity patterns[121]. Exercise-only interventions produce smaller energy deficits and therefore smaller amounts of weight loss in comparison to dietary restriction strategies. The following section reviews metabolic benefits specifically related to exercise training.

1.4.2 Beneficial effects of exercise training in obesity treatment

As discussed above, with greater adiposity there is a growing risk for ectopic fat accumulation and this is associated with IR. In obesity, increased intramyocellular lipid

content (IMCL) is attributed to a pathological imbalances between fatty acid availability and skeletal muscle oxidative capacity[122,123]. While weight loss through diet and/or exercise can reduce IMCL, skeletal muscle mitochondrial volume and oxidative capacity is only improved with the addition of exercise training[124]. In contrast to obesity-associated IMCL fat accumulation, it is worth noting that exercise trained individuals display increased IMCL, particularly in oxidative muscle fibres (type 1), but paradoxically have high levels of S_I[100]. In trained individuals, increased IMCL matches the higher fat oxidative capacity, especially during prolonged exercise[125] Adaptation to exercise training positively impacts on mitochondrial biogenesis and expression of enzymes of oxidative metabolism, which allows for the complete oxidation of fatty acid metabolites, and is linked to improved insulin sensitivity[126]. Greater increases in mitochondrial volume and oxidative capacity are achieved through regimes promoting higher exercise EE and are correlated to increased CRF[127]. This shows that in the trained state, the increased IMCL content is an adaptive response to exercise training rather than a pathological imbalance between fatty acid uptake and oxidation, as is found in the obese IR state[122]. In support of this adaptive response to exercise training, reduced-obese who exercised regularly were shown to have a metabolic profile similar to lean controls, while sedentary reduced-obese had a metabolic profile similar to obese controls[128]. Therefore, regular exercise facilitates metabolic improvements through its impact on mitochondrial volume, function and oxidative capacity, and over time should contribute towards reducing obesity-associated IR related to ectopic fat deposition. The following sections will now review evidence for changes in CRF, fat oxidative capacity, body composition and insulin sensitivity in response to exercise training interventions as summarised n Table 1 below.

Table 1.1: Summary of physiological changes in response to exercise training

Changes in response to exercise	Impact on whole body measurements	Effect on obesity outcomes
↑Blood volume ↑Capillary: muscle fibre density ↑Mitochondrial volume ↑Oxidative enzyme activity ↑Substrate availability	↑VO_{2peak} ↑Energy expenditure ↑Fat oxidative capacity	↓Body weight ↑FFSTM ↓fat mass ↑CRF ↑Insulin sensitivity

Note: VO_{2peak}: peak volume of oxygen consumption; FFSTM: fat free soft tissue mass; CRF: cardiorespiratory fitness.

1.4.2.1 Impact of exercise training on cardiorespiratory fitness

The CRF response to exercise training is conventionally determined as the change in VO_{2peak}, usually expressed per kg body weight, and is typically used as a marker of the success of exercise training interventions. As previously mentioned, it is determined by the capacity of the cardiovascular system to deliver oxygen to tissues and mitochondria for energy production during maximal exercise intensity[129]. The initial response to exercise training involves an increase in blood volume[130]. Subsequent improvements in cardiovascular capacity increases blood distribution to active muscles[130]. Capillary-to-fibre density is increased according to the muscle fibre mitochondrial volume and in line with the requirement for oxygen exchange[131]. These changes, together with increased mitochondrial volume and oxidative enzyme activity, improve skeletal muscle oxygen availability, extraction and utilization, all of which result in increased whole body VO_{2peak}[127,130–133].

Systematic reviews show that exercise training interventions, particularly those incorporating moderate-to-high intensity exercise, can achieve significant improvements in CRF and reduce cardiovascular disease risk and related lipid biomarkers, while also improving glucose control and insulin sensitivity[134–137]. While both high intensity exercise and moderate intensity endurance training improve CRF, there is an additional benefit from the incorporation of higher exercise intensity[138]. In slightly older populations (44.3±8.3 years), an increase of ~35% in VO_{2peak} was achieved through a 12 week program of track walking at an imposed higher intensity (83.2 ±8.0% VO_{2peak}). By comparison there was virtually no change in VO_{2peak} when the intensity was self-selected (73.8±9.2% VO_{2peak}), despite the self-selected group completing a greater overall walking distance[139]. Similarly, higher exercise intensity, rather than increased volumes of low intensity exercise, is necessary to improve CRF in sedentary obese populations[140]. Improved CRF was also shown in interventions using training intensities either at or above the lactate threshold. Here both groups improved CRF compared to controls, with those doing 60% of training above the lactate threshold showing enhanced CRF response compared to those working at the lactate threshold[141]. In contrast, in obese African American women with similar CRF, 14 weeks of high intensity interval training (HIIT: 4 bouts of 30-60 s at 75-90 %HRmax) increased exercise tolerance but did not improve CRF, despite high levels of adherence[142]. While moderate to higher intensity exercise training is required for CRF improvements, the HIIT format may not be an appropriate exercise modality in sedentary, obese populations with low baseline CRF[138,142], and may potentially impact on enjoyment, adherence and sustainability[143]. Exercise

interventions therefore need to take into account the optimal exercise dose (intensity and volume) required to achieve the desired results while also considering exercise modalities that are appropriate for the specific population group. Furthermore, in unsupervised conditions, in the absence of incentives, motivation and support, adherence to the exercise dose required to achieve improvements in CRF may be sub-optimal.

While many exercise intervention studies show an overall increase in average CRF compared to non-exercising controls, there is often high inter-individual response variability[140,144–148]. Some individuals may respond well to a period of exercise training, and are referred to as 'responders', while others show no improvement in CRF despite similar exercise training stimulus, and are termed 'non-responders'[149]. Many studies relate CRF non-response to inadequate duration, volume and intensity of the exercise training stimulus[140,147,150]. Six weeks of endurance training, exercising 4 or 5 times a week for 60 minutes showed an absence of CRF non-response compared to 69% non-response in groups exercising just once a week[150]. However, in this study, once the non-responders completed a further 6 weeks of exercise with 2 additional sessions per week, CRF non-response was eliminated[150]. At similar intensities, increasing exercise volume reduced CRF non-response by 50%, while at increased volume, increasing exercise intensity completely eliminated CRF non-response[140]. In considering VO_{2peak} response variability, most studies have considered exercise dose. While this undoubtedly plays a role, no studies have considered baseline variability in physiological characteristics, such as substrate utilization and fat oxidative capacity that may also contribute to variability in CRF response to exercise training.

Fat oxidative capacity may be a determinant of CRF response variability. Greater VO_{2peak} is associated with superior exercise performance[151] and is shown to correlate with higher fat oxidation rates[152]. A recent study also showed that during self-paced high intensity interval training at the same rate of perceived exertion, well trained athletes had similar blood lactate levels and rates of carbohydrate oxidation to recreational trained athletes, but had fat oxidation rates almost three times higher[153]. This study found that fat oxidation rates were highly correlated with VO_{2peak} and concluded that the increased capacity for high intensity work is largely explained by higher fat oxidation rates[153]. Whole body fat oxidation rates provide indirect information regarding mitochondrial capacity. Studies have shown that whole body fat oxidative capacity is associated with underlying mitochondrial fat oxidative capacity, which is increased in response to exercise training[151]. Similarly changes in whole

body fat oxidative capacity in response to exercise training reflect changes in mitochondrial volume, enzyme activity and oxidative capacity[151]. Despite the strong link between fat oxidative capacity and $VO_{2peak,}$ as well as capacity for high intensity exercise, to the best of our knowledge no studies have considered whether baseline variability in substrate utilization may impact on the subsequent CRF response variability to exercise training. A better understanding of this relationship could help to individualize the exercise dose and modality to improve CRF outcomes of exercise interventions.

1.4.2.2 Impact of exercise training on fat oxidation

Lower fat oxidation, identified in obesity and reduced with increasing BMI categories, has been causally associated with both increased IR and prospective weight gain[154,155]. Certainly sedentary overweight individuals have lower fat oxidation rates during submaximal exercise compared to normal weight controls[156]. However this may be related to levels of CRF and training status, and may be beneficially altered through exercise training. A study in recreationally-trained lean and overweight individuals, who were matched for CRF, showed similar fat oxidation rates over a range of exercise intensities[157]. Substrate oxidation in this study was consistently shown to be associated with CRF but not with body fat percentage[157]. This highlights that sedentary, overweight individuals demonstrate reduced fat oxidative capacity during submaximal exercise compared to lean counterparts, but that this can be improved through exercise training and increased CRF.

Exercise intervention studies have shown that moderate-to-high exercise training intensities are necessary, not only for improving CRF, but also for improving maximal fat oxidation during submaximal exercise[139]. As discussed above, the exercise stimulus essentially induces skeletal muscle adaptations including increased substrate availability, mitochondrial volume, function and oxidative capacity and this is reflected in whole body fat oxidative capacity[151]. Maximal capacity for fat oxidation in untrained populations is diminished with increasing body mass, but is generally improved in response to exercise training[151]. Earlier studies in normal weight sedentary women showed that moderate intensity exercise training (75% VO_{2peak}) reduced reliance on carbohydrate oxidation at the same absolute workload, while fat oxidation was increased at both the same absolute workload and relative intensity at post testing[158,159]. In sedentary, obese individuals, despite no improvement in CRF, HIIT reduced carbohydrate oxidation rates while maintaining fat oxidation rates at the same absolute

submaximal workload[142]. Therefore, in order to improve fat oxidation and reduce reliance on carbohydrate oxidation during submaximal exercise, it is important to incorporate moderate-to-higher intensity exercise, particularly among sedentary obese populations.

At rest, changes in substrate utilization in response to exercise training have been shown in some, but not all studies. In obese, sedentary populations, exercise intervention studies of both shorter (12-14 weeks)[142,160] and longer duration (16 months)[161], where no calorie restriction was applied, showed no change in resting RER despite reduced RER during submaximal exercise. This is surprising as cross-sectional studies show a distinct correlation between RER measured at rest and RER measured subsequently during submaximal exercise[162]. However, EE and substrate oxidation rates at rest are of significantly lower magnitude compared to the exercise state where skeletal muscle is recruited and energy production is significantly increased to fuel work. Resting RER is shown to be influenced by substrate availability (skeletal muscle and blood)[163,164] and is therefore highly influenced by day-to-day variability in energy and macronutrient composition of the diet and longer term dietary adaption, all of which result in altered available fuel stores[165]. In instances of calorie restriction, the replacement of glycogen stores is potentially submaximal, and thus fat must be mobilized and oxidized to meet the energy deficit, thereby increasing fat oxidation rates. Decreases in resting RER have been shown in shorter studies (7 weeks), where energy and carbohydrate intake were volitionally reduced[166], and where calorie restriction and weight loss (~8%) were incorporated[167]. While resting substrate utilization is of interest, substrate utilization during exercise is likely to be more relevant in assessing fat oxidative capacity of working muscle and the relative changes in response to exercise.

1.4.2.3 Impact of exercise training on body composition

Despite exercise training interventions showing only modest effects on weight reduction, body composition is improved through a loss of fat mass and gain in fat free soft tissue mass (FFSTM; predominantly skeletal muscle mass but also organ mass)[115]. VAT in particular, considered to play an important role in NCD presentation, is beneficially reduced through moderate-to-high intensity aerobic exercise training, even without weight loss[168]. Resistance training programs, while not associated with weight loss, do offer gains in skeletal muscle strength and mass[105]. In contrast, aerobic exercise and combined aerobic and resistance training have been shown to be effective at preventing weight gain[105] and improving body

composition[169]. Interestingly, in sedentary overweight women, HIIT was compared to continuous aerobic exercise training and found that while both improved work capacity, only continuous aerobic exercise showed reduced dual-energy X-ray absorptiometry (DXA) derived trunk and android fat measures[170]. Similar to the CRF response, high degrees of intra-individual variability are also shown in body composition responses to various exercise intervention modalities, with some individuals losing fat mass while others gain[115]. Highlighting the role of substrate utilization, studies show that higher maximal fat oxidation during exercise is associated with higher 24h fat oxidation and reduced or negative 24h fat balance, which would support fat mobilization and body composition improvements[171]. However, while substrate utilization and macronutrient balance may play an important role in body composition changes, there is currently very little research that directly links an individual's baseline capacity for fat oxidation in describing changes in body composition observed in response to exercise interventions.

1.4.2.4 Impact of exercise training on insulin sensitivity

Exercise training, particularly at higher intensities, is effective at improving blood glucose control in individuals at risk for T2DM[172]. A recent review on exercise training and its impact on insulin sensitivity has summarized findings from a number of exercise intervention studies[173]. They highlighted that exercise training increases skeletal muscle capiliarization and augments molecular remodelling that impacts on skeletal muscle uptake and utilization of glucose[173]. In addition exercise interventions regularly report on reductions in levels of systemic inflammation associated with obesity and reduced insulin sensitivity[174,175]. Even in the absence of improvements in CRF, aerobic exercise of higher intensity may also improve insulin sensitivity through reductions of lipid metabolites within skeletal muscle[173,176], potentially through improved mitochondrial capacity for complete oxidation fat[123]. Cross-sectional studies have also demonstrated that increased capacity for fat oxidation during submaximal exercise promotes negative 24h fat balance (indicating net 24h fat oxidation) and is associated with greater insulin sensitivity[171]. Certainly following bariatric surgery, individuals randomized to 6 months of moderate intensity exercise showed increased CRF, improved mitochondrial function, greater reductions in ceramide species associated with impaired insulin signalling and greater improvement in insulin sensitivity compared to non-exercising controls who underwent health education[177]. Resistance training also improves insulin sensitivity, possibly related to increased FFM[178,179] as well as specific adaptions to

different fibre types (glycolytic type 2a versus oxidative type 1 fibres), and suggests that the combination of aerobic and resistance exercise training may provide additive benefits for improving insulin sensitivity[173]. Evidence therefore supports a possible link between improved fat oxidative capacity and increased insulin sensitivity in response to exercise training.

1.5 Weight-loss maintenance

Successful weight-loss maintenance has been described as a 10% reduction of initial body weight, that is subsequently maintained for a period of at least 1 year[180]. However, weight loss is not only difficult to achieve but also to maintain. An estimated 53% of Americans are currently attempting weight loss, with a further 25% struggling to maintain their reduced weight[181]. Typically only 20% of overweight and obese individuals with a BMI $\geq$ 27 $kg.m^{-2}$ will achieve successful weight loss, while around 35% of those attempting weight loss actually gain additional weight[182]. Further, around 80% of weight loss will be reversed over the following 5 years, particularly when weight loss is more moderate (less than 10% of initial body weight)[183]. Other studies show that around 17% of individuals successfully achieving weight loss will maintain just 10% of this weight reduction for a period longer than one year, with around 37% maintaining just 4.4%[184]. In obesity treatment it is therefore important to explore the adaptive physiological changes accompanying weight loss, that impact on EI, TDEE and macronutrient balance and hence predispose individuals to weight regain.

This section starts with an overview of the physiological adaptions to weight loss that act on both sides of the energy balance equation and create an 'Energy Gap' that effectively defends the previously higher body weight set point alluded to above. The following subsections then review this adaptive response to weight loss in more detail, specifically investigating changes in TDEE and substrate utilization (specifically fat oxidative capacity) that may predispose weight-reduced individuals to weight regain.

1.5.1 Physiological compensation affecting energy balance – The Energy Gap

King et.al. (2007)[185] described the behavioural and physiological compensatory responses to an induced energy deficit. He considered these compensatory responses to be automatic

(biologically inevitable) or volitional (intentional). An automatic compensatory response can manifest in a behavioural response, for example through increased sedentary behaviour (in response to perceptions of fatigue) or increased food intake (in response to increased appetite) following exercise. These may be driven by physiological responses including changes in appetite related hormones or a biological decrease in resting metabolic rate RMR. In contrast, volitional compensatory responses are by their nature behavioural, and are therefore entirely self-selected. However, it is likely that the physiological compensatory responses, acting to restore energy balance, will have a modulatory effect on the individual's volitional compensatory responses and therefore play a substantial role in weight regain [186]. Combatting these physiological compensatory responses is considered to present one of the major challenges to obesity treatment [187].

Table 1.2 below summarizes the physiological factors implicated in limiting successful weight loss and predisposing individuals to weight regain. The hypothalamus is primarily involved in homeostatic regulation of energy balance, receiving signals related to short term food intake, as well as changes in long term energy stores[188]. This information is integrated and fed forward via neurons to higher areas of the brain and back to the periphery to influence both EI and EE[188]. With weight loss, reductions in specific hormones (e.g. leptin and insulin) centrally signal reduced energy stores[187]. Reduced food intake as a result of calorie restriction and more rapid clearance of nutrients (glucose and fatty acids) from the blood stream due to improved insulin sensitivity, further signal reduced nutrient/substrate availability[187]. Appetite hormones released from the gut that centrally signal hunger (e.g. Ghrelin), are increased during energy deficit and weight loss, while those signalling satiety following food ingestion (peptide$_{3\text{-}36}$ YY, glucagon-like-peptide-1 and cholecystokinin) are reduced[33,189,190]. Together these afferent signals to the hypothalamus are either fed forward, favouring increased energy intake to replenish energy stores, or initiate efferent signals to the periphery to increase energy efficiency and reduce EE[187]. Reductions in body mass also reduce the energy cost of general activity, while reduced FFSTM leads to declines in RMR[187]. Dietary restriction, particularly severe calorie restriction, is associated with increased physiological stress which transiently increases hypothalamic-pituitary-adrenal (HPA) axis activation[190–192]. This results in elevated circulating cortisol particularly in the initial phase of weight loss which in turn increases lipolysis but also appetite[190–192]. Reduced sympathetic and increased parasympathetic activation occurs in response to weight loss, evidenced by reductions in urinary catecholamines, muscle sympathetic nerve activity and

plasma renin activity[193,194], further contributes to increased appetite and reduced EE and fat oxidation, thus facilitating increased fat storage[190,191]. Thyroid hormone levels may also be reduced in response to reduced sympathetic activation following weight loss[195], leading to reductions in components of EE as well as increased fat storage[190,191]. Furthermore, there is evidence that non-protein RER is raised during weight-loss maintenance and during periods of weight regain, suggesting a preference for carbohydrate oxidation and fat storage, which may also drive an increase in appetite and food intake[196]. Therefore, with successful weight loss, a number of physiological adjustments occur, favouring an increase in energy intake, a reduction in components of TDEE, a diminished capacity for fat oxidation and increased capacity for fat storage.

Table 1.2: Summary of the changes that accompany weight loss

Changes accompanying weight loss	Physiological impact	Overall impact
↓Leptin	↑appetite	
↑Insulin sensitivity; ↓insulin	↑energy efficiency, ↓TDEE	**Energy Balance**
↑Ghrelin	↑hunger → overconsumption	Increased body weight
↓PYY, CCK	↓satiety → overconsumption	(↑EI; ↓TDEE)
↑Energy efficiency	↓TDEE	
↓FFSTM, ↓FM		**Macronutrient Balance**
↑HPA activation (↑Cortisol)	↑appetite & ↑lipolysis	Increased FM
↓sympathetic activation	↓EE; ↓fat oxidation; ↑fat storage	(↓fat oxidation ;↑fat storage)
↑parasympathetic activation	↑EI; ↑CHO oxidation	
↓Thyroid hormones	↓EE; ↑fat storage	

Note: this table summarizing changes that accompany weight loss has been adapted from previous review articles as referenced above[187,190,197,198]. PYY: Peptide YY; CCK: cholecystokinin; FFSTM: fat free soft tissue mass; HPA: hypothalamic pituitary adrenal axis; TDEE: total daily energy expenditure; EE: energy expenditure; EI: energy intake; CHO carbohydrate; FM: fat mass.

1.5.2 Physiological compensation affecting energy expenditure

The reduction in TDEE that accompanies weight loss is shown to be in excess of that described by weight loss-associated changes in body composition, and has been termed '*adaptive thermogenesis*'[198]. Early studies in obese and never obese groups found that maintaining a body weight 10% above or 10% below the normal body weight resulted in a compensatory increase of 16% or a decrease of 15% in TDEE (adjusted for body composition), respectively[46]. These compensatory changes affected all components of relative energy expenditure and suggested a mechanism to return to the usual body weight[46]. As discussed above, this mechanism may involve reduced circulating levels of the adipocyte-derived hormone leptin, as a result of reduced FM, that influence changes in sympathetic

activation and thyroid function[191,199–201]. Declines in leptin may thus need to be managed during dynamic weight loss to prevent the energy gap from widening and limiting weight loss[202]. TEF is similarly shown to be lower in formerly obese compared to never obese subjects, and this may also form part of the adaptive response to weight loss[203]. Furthermore, besides a reduction in spontaneous physical activity, a 10% weight reduction has also been shown to result in a 20% increase in skeletal muscle efficiency, which further reduces activity associated EE[191]. A previously obese, weight reduced individual could thus require a far lower daily calorie intake than a never obese individual matched for body weight and composition, which has implications for dietary prescription in weight loss maintenance[191].

1.5.2.1 Adaptive thermogenesis in acute weight loss and weight-loss maintenance

Calorie restriction, together with the amount of weight lost, negatively impacts on TDEE, particularly during the acute weight loss phase. Studies show that during the first 10% of weight loss, the reduction in TDEE significantly impacts both RMR and non-resting EE (NREE = TDEE – RMR), thereafter further weight loss is predominantly associated with additional declines in NREE [201]. Therefore, exercise in combination with dietary restriction may be a preferred approach to creating the required energy deficit without excessive dietary restriction, and potentially attenuating declines in NREE. This was elegantly demonstrated in a study that created an energy deficit of 25% of energy requirements either entirely through calorie restriction (CR) or through 12.5% dietary calorie restriction combined with 12.5% increased exercise EE (CR + Ex). The CR and CR + Ex showed similar weight loss (-10.4±0.9% versus -10.0±0.8%) and body composition changes and both groups showed similar metabolic adaption, as measured by sedentary 24h EE and sleep EE[204]. However, after adjusting for sedentary 24h EE, TDEE decreased in the CR group by 316 ±118 kcal/d at month 6 (through reduced habitual and voluntary physical activity), whereas the CR + Ex showed no changes in these measures[205]. Therefore, while an energy deficit created either through diet or a combination of diet with exercise will result in similar metabolic adaptation, incorporating exercise into the weight loss strategy helps to maintain and prevent declines in NREE.

The degree of energy deficit and the rate and scale of weight loss are important factors in determining adaptive thermogenesis in response to acute weight loss, even if metabolically active FFSTM is preserved. One study in morbidly obese participants assigned to an energy

restricted diet plus vigorous physical activity over a 30 week period, showed that individuals achieved reductions of around 38% in body weight, with only 17% of that being a loss of FFM[206]. Despite preventing major loss of FFM and a significant increase in energy expenditure on physical activity, RMR at week 30 was 504 ±171 kcal/d lower than that predicted by changes in body composition[206]. The data from the same group of participants was subsequently compared to individuals who achieved similar weight loss following gastric bypass surgery. Results showed that compared to the bariatric surgery group, the diet and exercise group had maintained a significantly greater proportion of FFM, mainly through the incorporation of resistance exercise, but also through increased protein intake, but nonetheless showed greater metabolic adaption[207]. This study also found that adaptive thermogenesis was associated with the degree of energy imbalance, rate of weight loss and change in circulating leptin levels[207]. These findings thus suggests that with such severe calorie restriction ,the body responds as if to starvation, a major threat to survival, with a significant reduction in RMR and a widening of the energy gap.

Adaptive thermogenesis in longer term weight loss maintenance is controversial. One study compared weight matched individuals at usual weight, after recent weight loss (5-8 weeks) and after maintained weight loss of over 10% (>1 year)[208]. This study showed that individuals who had both recent and sustained weight loss had lower TDEE, RMR and NREE compared to individuals at usual weight, based on predictive equations determined using age and body composition[208]. Conversely other studies show that energy restriction results in a 'hypothyroid hypo-metabolic state' during dynamic weight loss that returns to normal once energy balance and weight stability has been restored[209]. As shown above, reduced circulating levels of leptin following calorie restricted weight loss have been shown to be a significant and independent determinant of this metabolic adaption, and levels may remain reduced in weight maintenance[210].

Lifestyle factors such as diet and exercise also have an important impact on TDEE with implications for maintaining reduced weight and preventing weight regain. Low fat diets result in the greatest reduction in RMR in response to weight loss[211], while diets higher in protein with an emphasis on low glycaemic load have shown less of a decline in RMR[212]. Incorporating higher levels of EE on physical activity during a weight loss intervention is also shown be associated with more stable body weight and lower weight regain at 6 month and 12 month follow-up, emphasizing the importance of incorporating physical activity

during weight loss and weight loss maintenance [213–215]. This would seem to suggest that an optimal strategy for weight loss maintenance would involve achieving energy balance at the reduced weight, maintaining dietary vigilance, optimizing macronutrient intake, maintaining FFSTM and increasing levels of physical activity. While adaptive thermogenesis in weight loss maintenance is controversial, small numbers of individuals are able to successfully maintain reduced weight. It remains unclear however whether these adaptive responses remain present in long term weight loss maintenance and if so, what strategies are these individuals employing to underpin their success. Better understanding of these factors will help to inform both dietary and lifestyle prescription for the weight maintenance phase to prevent weight regain.

1.5.2.2 Physiological compensation affecting substrate utilization

In terms of substrate utilization, post-obese individuals who have lost weight through dietary calorie restriction alone, show suppressed fat oxidative capacity and a preference for carbohydrate metabolism during submaximal exercise compared to weight matched controls[154]. Studies also show that at long term follow-up (>4 years), women maintaining weight reductions greater than 10% of initial body weight exhibited higher maximal fat oxidation rates than individuals maintaining more moderate weight loss[216]. Nevertheless, post-obese individuals show significantly greater efficiency in the uptake and storage of fat compared to matched controls with no weight loss history[217]. Furthermore, weight loss in the absence of exercise training, improves insulin sensitivity and metabolic flexibility but does not increase mitochondrial content and fatty acid oxidative capacity[218]. However, this might equally be countered by maintaining adequate levels of physical activity in weight loss maintenance. With dietary relapse, more efficient uptake and storage of fat, rather than oxidation, could therefore increase risks for gains in body weight and FM [187]. Clearly there appears to be a lower capacity for fat oxidation that accompanies obesity that may remain present in weight loss maintenance, potentially leaving individuals susceptible to weight regain.

1.6 Conclusion

In summary to this literature review, strategies are required to address the increased health risks associated with the obesity epidemic. The development and maintenance of obesity is increasingly shown to be the result of dysregulated energy homeostasis involving persistent positive energy balance and defence of a higher body weight set point. In addition to excess EI, low levels of TDEE support a positive energy balance and prospective weight gain. Reducing sedentary behaviour and increasing PAL may improve the ability to more consistently match EI with EE, and is an important consideration in the treatment of obesity and the associated risk factors. Energy and macronutrient balance also affects energy stores and partitioning, with low fat oxidative capacity favouring fat storage and increasing adiposity. High reliance on carbohydrate oxidation and defence of glycogen stores potentially drives increased appetite, suggesting a role in weight and fat mass gains over time. As obesity becomes established, dysregulated energy partitioning and increased ectopic fat deposition increasingly result in IR and metabolic inflexibility, implicating reduced fat oxidative capacity in their aetiology. Obesity treatment should therefore consider interventions that target these metabolic complications, including calorie restriction and physical activity.

1.7 Aims and Objectives

Prevention of weight gain is a primary goal in reducing obesity prevalence and progression, while in obesity treatment, even small amounts of weight loss can have significant health benefits. Weight loss interventions employing dietary calorie restriction alone result in more weight loss compared to exercise only interventions. However, exercise training improves CRF which is independently associated with reduced risk for T2DM, cardiovascular disease and mortality. It is also shown to improve metabolic flexibility, body composition and insulin sensitivity and is instrumental in preventing weight gain. Given the beneficial effects of exercise training in improving CRF, fat oxidative capacity, body composition and insulin sensitivity it is reasonable to expect that exercise training would confer beneficial effects on high risk population groups.

Obesity treatment ultimately involves weight reduction. However, the success of weight loss and weight loss maintenance is poor. Adaptive physiological changes accompanying weight loss, impact on energy and macronutrient balance. These changes limit the success of weight

loss and weight loss maintenance, predisposing individuals to weight regain. Combatting these physiological compensatory responses is considered to present one of the major challenges to obesity treatment. Small numbers of individuals are able to successfully maintain reduced weight. However, these adaptive responses in weight loss maintenance are controversial and it is unclear whether metabolic rates, EE, substrate utilization and insulin sensitivity in long term successfully weight reduced individuals return to levels that are indistinguishable from phenotypically similar individuals without a weight loss history. Part 2 of this thesis then focuses on establishing whether a history of weight loss/weight loss relapse alters metabolic rate, substrate utilization and insulin sensitivity such that these individuals are predisposed to weight gain/regain.

1.7.1 Part 1 – Exercise Training Intervention

Evidence presented suggests that exercise training provides a vehicle for preventing weight gain and achieving modest weight loss. An exercise intervention, incorporating moderate-to-high intensity exercise, may also lead to improvements in CRF, fat oxidative capacity, body composition and insulin sensitivity. Black SA women show the highest prevalence of obesity in sub-Saharan Africa (42%)[15] and despite studies showing that a large proportion of this population group are classified as sufficiently active (although not distinguishing between moderate and vigorous activity)[219], they display very low levels of CRF[220]. The activity profile of black SA women also shows that they predominantly engage in lower intensity, ambulatory activity related to transportation, but do little or no leisure related moderate-to-vigorous activity[219]. They also demonstrate high levels of sedentary behaviour. Consequently longitudinal studies in this group have highlighted increases in body weight and measures of body fatness over 5 year follow-up, irrespective of habitual activity levels[219]. Compared to their white counterparts they also demonstrate physiological differences in terms of body fat distribution, with greater gluteal FM and reduced VAT. Furthermore, gluteal fat depots in black SA women show differential gene expression compared to white women, pointing to greater risk for adipocyte hypertrophy[87], reduced vascularization[89] and increased hypoxia and inflammation[90], which was in turn associated with increased IR. Studies also show that black SA women have increased peripheral insulin resistance relative to BMI-matched white women, placing them at greater risk for T2DM in later life[221,222]. Despite low CRF, high prevalence for obesity and increased risk for T2DM[223], no formal exercise intervention studies have previously been carried out specifically in black SA women.

In Part 1 of this thesis I hypothesised that metabolic rate and substrate utilization in a sedentary obese population will be beneficially altered through exercise training, and that these changes would be associated with improvements in CRF, body composition and insulin sensitivity. The aims of part 1 were therefore to assess the effects of a 12-week exercise intervention on changes in CRF, metabolic rate and substrate utilization, at rest and during exercise, along with changes in body weight and body composition in obese black SA women. The results of Part 1 are presented in two separate chapters. In chapter 2, I compared changes in CRF, metabolic rate and substrate utilization, at rest and during exercise, along with changes in body weight and body composition between exercise participants and non-exercising controls. In chapter 3, I firstly explored the inter-individual variability in CRF response to the intervention and then examined the correlates of the inter-individual variability in CRF response, comparing differences in metabolic rate and substrate utilization at rest and during exercise, along with changes in body composition and insulin sensitivity, between low and high CRF responders. The relationships between substrate utilization and CRF response, as well as insulin sensitivity and body composition response were also explored. These results provide important evidence for the efficacy of exercise training in reducing health risks in this high risk population group.

1.7.2 Part 2 – Weight Loss Maintenance

Obesity is accompanied by substantial metabolic dysregulation, evidenced through reduced fat oxidative capacity and increased levels of peripheral insulin resistance. With weight loss, despite metabolic improvements, weight reduced individuals may display adaptive thermogenesis and reduced fat oxidative capacity, which together may predispose them to weight regain. Therefore it is of interest to study individuals who have successfully maintained weight loss in order to explore whether these individuals continue to show evidence of adaptive thermogenesis and reduced fat oxidative capacity, compared to BMI-matched controls without a history of weight loss. Furthermore, while weight loss improves insulin sensitivity, it is not known whether this returns to levels comparable to individuals without a weight loss history when weight loss is maintained over time. Additionally, it is of interest to explore the strategies employed by long term successfully maintained weight reduced individuals as this may inform future weight maintenance programs, as well as lifestyle interventions aimed at preventing weight gain. Among weight loss relapsed

individuals it is also important to explore whether the history of weight cycling has an impact on measures of metabolic rate, substrate utilization and insulin sensitivity compared to individuals who have never attempted weight loss.

In Part 2, I hypothesized that metabolic rate and substrate utilization, along with insulin sensitivity will be altered through weight loss/regain, predisposing these individuals to prospective weight gain/regain, thereby impairing successful weight loss maintenance. Part 2 therefore aimed to examine differences between long term, successfully maintained weight reduced and weight loss relapsed individuals compared to BMI-matched controls with no weight loss history. Specifically it aimed to explore differences in metabolic rate, substrate utilization and insulin sensitivity that may occur as a result of prior weight loss history, potentially predisposing these individuals to future weight gain compared to phenotypically similar individuals with no weight loss history. I also compared physiological and behavioural differences (dietary intake and physical activity) between experimental and control groups. By understanding these differences it may be possible to develop strategies to counteract these adaptive changes to weight loss to support successful weight loss, as well as weight loss maintenance.

CHAPTER 2

2. Higher baseline fat oxidation promotes gynoid fat mobilization in response to a 12 week exercise intervention in sedentary, obese black South African women.

Accepted for publication:

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For this study I was responsible for: developing the research questions; advertisement and recruitment of participants; carrying out the graded exercise test to determine VO_{2peak}; carrying out the steady state exercise testing; carrying out the resting measurements; collection and analysis of dietary data; statistical analysis and interpretation and write-up of the manuscript.

2.1 Introduction

Low levels of physical activity and cardiorespiratory fitness (CRF) are strongly associated with increased metabolic risk and obesity[25,224,225]. Obesity contributes to dysregulation of endocrine and neural pathways involved in fat deposition and mobilization[226]. Sedentary obese individuals exhibit lower maximal rates of fat oxidation during exercise compared to lean counterparts[151]. Furthermore, a low ratio of fat-to-carbohydrate oxidation has also been associated with subsequent increases in body weight[65,227] as well as fat mass (FM)[66]. The respiratory quotient-food quotient model asserts that energy balance and weight stability is achieved when macronutrient balance is also achieved[58]. Carbohydrates have limited storage capacity and have oxidative priority over fat; such that over the short term, balance between carbohydrate intake and oxidation is generally achieved[58]. However positive energy balance, coupled with obesity-related declines in fat oxidative capacity, are likely to promote positive fat balance, driving the increased storage of dietary fat[55]. Investigating potential strategies to improve both CRF and fat oxidative capacity is therefore important in the prevention and treatment of obesity.

While calorie-restricted weight loss does not necessarily restore fasting fat oxidation, exercise training can improve fat oxidation at rest and during submaximal exercise[109,167]. Thus, even in the absence of weight loss, combined aerobic and resistance training supports reductions in FM, particularly from abdominal and visceral fat depots[228–230]. However fat oxidative capacity and its impact on body weight and composition have been shown in some[141,166,176,231–233] but not all studies[169,234], with exercise intensity playing an important role in the desired outcome. From the literature it appears that moderate-high intensity, combined resistance and aerobic exercise may be an appropriate vehicle for improving CRF, fat oxidative capacity and body composition.

South Africa (SA), and other geographical locations including the United States of America (USA) have shown that black African women have a very high prevalence of obesity and type 2 diabetes [20,42,235,236]. While black African women commonly have less central and more peripheral (lower-body) FM compared to BMI-matched white counterparts, a feature thought to be protective, they are more insulin resistant, hypersecreting insulin in order to maintain normoglycaemia [85,89,221,237–239]. Longitudinal studies from SA also show that this population group is at risk for weight gain and increasing obesity over time [219]. Within SA, the activity

profile of black African women indicates substantial engagement in low intensity, ambulatory activity (walking for transport), but little or no moderate-to-vigorous intensity physical activity and high rates of sedentary behaviour [219,240]. Consequently CRF is very low [15,219,220,240]. Exercise training would therefore offer an attractive vehicle to improve CRF and promote improvements in body weight and composition in this high risk population group. However, to date no supervised exercise training interventions have been conducted in obese, black SA women.

We hypothesized that a structured exercise training intervention in obese black SA women would increase CRF, metabolic rate and fat oxidation at rest and during submaximal exercise. We further hypothesized that greater fat oxidative capacity at the outset would facilitate improvements in body composition in response to exercise, particularly in visceral abdominal depots associated with reduced insulin sensitivity. Therefore, this study aims to examine the impact of a 12 week exercise intervention on changes in CRF, EE and substrate utilization (at rest and during exercise) in obese black SA women and to explore the associations with changes in body composition.

2.2 Methods

The full research protocol for all procedures and baseline characteristics of the cohort have been previously described in detail[241].

Participants

Forty-five black SA women were recruited from the Western Cape, SA and randomized into an exercise (n=23) on control group (n=22). Inclusion criteria were: 20-35 y, obese (BMI 30-40 kg/m^2), weight stable for 6 months, black SA (both biological parents *isiXhosa*), sedentary (not >1 exercise session of >20 min/week over previous 12 months), no diagnosed disease, not on medication, no surgical procedures in the previous 6 months and on injectable contraceptive (depot medroxyprogesterone acetate, 400 mg, >2 months). HIV and pregnancy tests were completed to ensure participants were HIV negative and not pregnant. Of the individuals recruited, 10 withdrew (3 from the exercise group and 7 from the control group). Of these, 9 withdrew due to time constraints and 1 due to pregnancy. The analysis includes 35 individuals, 20 in the exercise group and 15 in the control group. Given the participant burden in terms of time and travel requirements participants were reimbursed at an hourly

rate in accordance with recommendations from the Health Sciences Human Research Ethics Committee at the University of Cape Town. This study was approved by the Human Research Ethics Committee at the University of Cape Town (HREC REF: 054/2015) and conducted in accordance with the 1964 Helsinki declaration and later amendments. Participants provided written consent prior to testing. The trial was registered retrospectively with the Pan African Clinical Trial Registry database (PACTR20171102789113).

Study design

Thirty-five participants completed the study (CTL, n=15; EXE n=20)[241]. EXE undertook 12 weeks of aerobic and resistance exercise, while maintaining usual dietary patterns. CTL continued habitual activity and dietary patterns. All participants attended three pre- and post-testing sessions (See Figure 2.1 below). At the first session, anthropometry was measured and body composition was determined by dual energy x-ray absorptiometry (DXA). A treadmill-based graded exercise test was performed to determine peak oxygen consumption (VO_{2peak}). At session two, a treadmill-based submaximal exercise test at 50% VO_{2peak} was completed. At least 72 h thereafter, participants were provided a standardized evening meal and fasted overnight (10-12 h), remaining at the facility. The following morning (06:00 h), resting metabolic rate (RMR) (40 min) was measured. At weeks 0, 4, 8 and 12 dietary data and objectively measured physical activity data was collected.

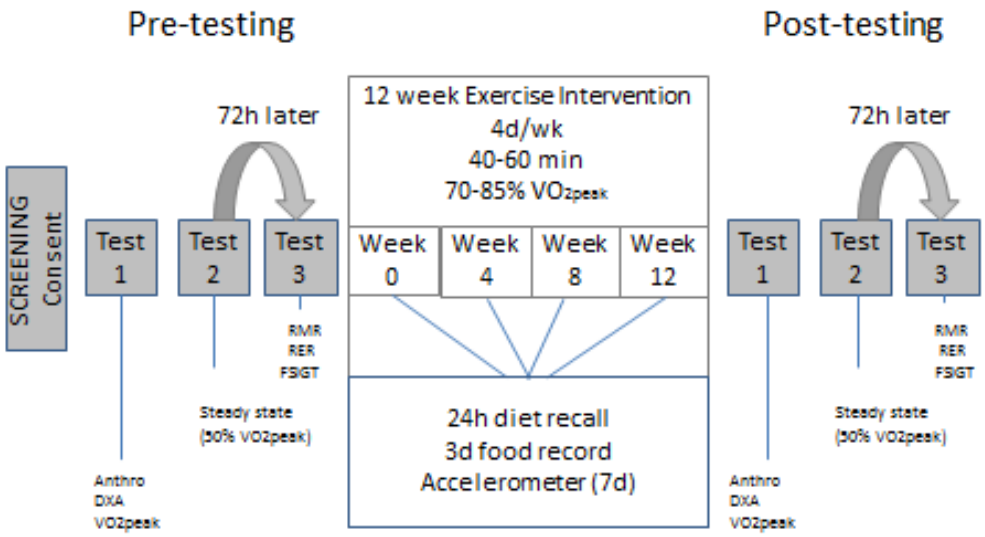

Figure 2.1 – Study design and testing
Note: Anthro: anthropometry; DXA: body composition measurement using dual energy x-ray absorptiometry; VO_{2peak} : peak volume of oxygen consumption; RMR: resting metabolic rate; RER: respiratory exchange ratio; FSIGT: frequently sampled intravenous glucose tolerance test.

Exercise intervention

The 12-week exercise intervention incorporated aerobic and resistance training at moderate-vigorous intensity (70-85% peak heart rate, HR_{peak}) progressing from 40 to 60 minutes per session, 4 days per week, supervised by a trained human-movement and exercise specialist. Aerobic exercise (>75% HR_{peak}) included dance- and boxing-related exercise, running, skipping and stepping. Resistance exercise (60-70%HR_{peak}) included press-ups, shoulder press, bicep curls, squats and lunges using body weight, resistance bands and free weights. Heart rate monitors (Polar A300, Kempele, Finland) were worn and monitored during all training sessions to ensure adherence to the prescribed exercise intensity.

Body Composition

Weight (BW-150, NAGATA, Tainan, Taiwan) and height (3PHTROD-WM, Detecto, Missouri, USA) were measured in lightweight clothing. Waist (WC, at the umbilicus) and hip (HC, at the largest protrusion of the buttocks) circumferences were measured using a metal anthropometric tape measure (*CESCORF*, Brazil). Whole body composition including fat mass (FM) and fat-free soft tissue mass (FFSTM) were measured using DXA (Discovery-W®, version 12.7.3.7, Hologic, Bedford, MA, USA) according to standard procedures. Sub-total (excluding the head) FM and FFSTM were used for all analyses. DXA-derived regional body fat distribution, including android, gynoid, trunk, subcutaneous (SAT) and visceral (VAT) adipose tissue, were measured as previously described[242]. Android, gynoid and trunk FM are expressed in kilograms and as a percentage of subtotal FM.

Graded exercise test

A walking, treadmill-based (C, Quasar LE500CE, HP Cosmos, Nussdorf-Traunstein, Germany), graded exercise test was performed measuring pulmonary gas exchange (Cosmed Quark CPET, Rome, Italy) to determine VO_{2peak} and respiratory exchange ratio (RER). Participants were familiarized to the equipment prior to testing and wore a heart rate monitor to determine HR_{peak}. The first 6 min used a modified Bruce protocol as a warm up and to obtain three stages of steady state metabolism. Subsequently VO_{2peak} was obtained using an adapted ramp protocol[243] until volitional exhaustion.

Steady State Testing

Following an overnight fast (10-12 h) EE and RER were measured at 50% VO_{2peak} ($ml.min^{-1}.kg^{-1}$)[244,245] using indirect calorimetry (Cosmed Quark CPET, Rome, Italy). Workload (treadmill gradient) was adjusted during the first 5 minutes to achieve the required exercise intensity. Thereafter participants walked for 10 min at the selected workload, measuring EE, RER and HR and the mean of the 10 min test is reported. Steady state post-testing was repeated at an absolute workload (same workload as pre-intervention) and relative intensity (50% of post-intervention VO_{2peak}), with 10 min recovery between[246,247].

Resting Metabolic Rate

Resting EE and RER were measured following an overnight fast (10-12 h, >72 h after VO_{2peak} testing). The evening prior to the test, participants consumed a standardized meal (representative of a typical evening meal) at 20:00 h (Energy: 2,456 kJ, Protein: 21 g (14%), Carbohydrate: 49 g (33%) and Fat: 32 g (48%)) remaining at the facility and abstaining from alcohol or caffeine. The following morning (6:00 h) participants remained awake in the supine position in a quiet, temperature controlled (21-24□C) room. Basal EE and RER were recorded for 40 min, using the ventilated hood technique (Cosmed Quark CPET, Rome, Italy). Measurements were based on the mean of the last 30 min[248].

The metabolic cart was calibrated before all tests using a 3 L syringe and analysers using standard gas mixtures of room (atmospheric) air (20.95% O_2, 4% CO_2 and the balance Nitrogen) and calibration gas (16% O_2, 4% CO_2 and the balance nitrogen; Air Products, Cape Town, South Africa). Total rates of fat and carbohydrate oxidation were calculated using the equations of Weir and Frayn[249,250] and are reported per kg FFSTM.

Dietary Intake

At pre-testing and every 4 weeks thereafter a 24 h dietary recall was completed with a registered dietitian. Thereafter 3 d food records were completed in the same format, including one weekend day. On return, the dietitian checked the records for completeness, clarifying incomplete records with participants. Dietary intake was calculated as the average daily intake from the 24 h recall and 3 d food record[251,252]. Nutrient intake analysis was calculated by the Biostatistics Research Unit of the South African Medical Research Council (Parow, Western Cape, South Africa) using the South African Food Composition Database System

(SAFOOD, the South African Food Composition Database, Version 2016, Parow, Cape Town, South Africa)[253].

Physical Activity

Physical activity is reported as a daily step count and measured using accelerometers (ActivPAL, activPAL3c, PAL Technologies Ltd, Glasgow, UK) at pre-intervention, 4, 8 and 12 weeks. The activPAL was attached to the mid anterior right thigh and worn continuously for 7 days. Data was analysed using the activPAL software (PAL Technologies, version 7.2.32, Glasgow, UK).

Statistical Analysis

Statistical analysis was carried out using Stata (Version 12, Stat Corp, College Station, Texas 77845, USA). Data was assessed for normality using the Shapiro Wilks test. Mean and standard deviations (SD) were reported for normally distributed data and median and inter-quartile-ranges (IQR) for non-parametric data. Mixed models with repeated measures were used to compare differences in the changes in outcome variables between groups over time (group, time and group-by-time interaction effects). Post-hoc tests informed differences between groups at either baseline or post-testing as well as within group changes over time. Pearson and Spearman correlation coefficients were used to examine associations and multiple linear regression models were used to model predictors of change in secondary outcome variables. The multiple linear regression models were tested for normality of residuals, linearity and homoscedasticity. Outliers were checked for influence and leverage and multicollinearity of predictors was assessed using the variance inflation factor (VIF > 5). For all tests, significance was accepted at $p<0.05$.

2.3 Results

There were no differences in age between CTL (23 y; IQR 21-27) and EXE (22 y; IQR 21-24, p=0.748). EXE completed 38.1±6.3 (range: 25 – 49, 79.4%) of the 48 exercise sessions at a mean intensity of 79.7±4.0% HR_{peak} (range: 71 – 85% HR_{peak}).

Body composition

Baseline measures of body weight, anthropometry and body composition were not different between EXE and CTL ($p>0.05$, Table 2.1). There were interaction effects for weight, BMI,

WC, HC and WHR ($p<0.05$). Post hoc tests showed that weight, BMI, WC and HC decreased in EXE in response to exercise training (post hoc Δtime: $p=0.038$, $p=0.029$, $p<0.001$ and $p=0.005$, respectively), while in CTL, weight, BMI and WC increased (post hoc Δtime: $p=0.030$, $p=0.038$ and $p=0.016$). There were interaction effects for gynoid FM (kg: $p=0.017$ and %FM: $p=0.002$). As a percentage of FM, gynoid FM (%FM) decreased in EXE in response to the exercise intervention (post hoc Δtime: $p<0.001$). In contrast, there were no changes in DXA-derived measures of FFSTM, FM, android FM, abdominal SAT or VAT in either EXE or in CTL. However, over the 12 week period, gynoid FM increased in CTL (post hoc Δtime: $p=0.031$), but as a percentage of FM this was unchanged (post hoc Δtime: $p=0.323$).

Table 2.1 –Body composition and body fat distribution

	CTL (n=15)		**EXE (n=20)**		**P values**		
	Pre	Post	Pre	Post	Group x Time	Group	Time
Anthropometry							
Weight (kg)	**87.8±10.9**	**88.8±11.0***	**84.1±8.7**	**83.3±9.7*$^{\varphi}$**	**0.003**	0.267	**0.030**
BMI (kg.m^{-2})	**33.4±2.7**	**33.8±2.8***	**34.1±2.8**	**33.8±3.1***	**0.003**	0.43	**0.038**
WC (cm)	**103.4±8.1**	**105.9±9.5***	**103.6±7.4**	**100.4±8.6*$^{\varphi}$**	**<0.001**	0.927	**0.016**
HC (cm)	117.5±1.6	**117.9±1.6**	**114.5±1.4**	**112.7±1.4*$^{\varphi}$**	**0.023**	0.153	0.548
WHR	0.88±0.05	0.90±0.07	0.91±0.07	0.89±0.06	**0.016**	0.193	0.094
DXA-derived measures of body composition$^{(\#)}$							
FFSTM (kg)	38.4 (35.0-40.7)	38.2 (35.4-38.2)	37.1 (33.6-39.6)	37.1 (33.9-39.9)	0.353	0.226	0.217
FFSTM (%)	47.6±4.2	47.5±3.8	47.8±2.7	47.9±3.1	0.548	0.840	0.543
FM (kg)	40.8±7.0	41.2±6.2	38.6±5.5	38.6±6.7	0.189	0.302	0.076
FM (%)	50.4±4.3	50.4±3.9	50.2±2.7	50.1±3.1	0.521	0.912	0.518
Android FM (kg)	3.3±1.0	3.3±1.0	3.2±0.5	3.1±0.6	0.388	0.562	0.818
Android (%FM)	8.0±1.3	7.9±1.5	8.3±1.0	8.1±1.0	0.860	0.572	0.163
Gynoid FM (kg)	**7.8±1.1**	**8.0±1.0***	7.1±1.3	**7.0±1.4$^{\varphi}$**	**0.017**	0.085	**0.031**
Gynoid (%FM)	19.4±2.3	**19.6±2.3**	**18.5±1.7**	**18.2±1.6*$^{\varphi}$**	**0.002**	0.129	0.323
SAT (cm^2)	533±100	533±09	529±74	523±81	0.535	0.895	0.937
VAT (cm^2)	130±46	128±37	137±28	132±28	0.509	0.505	0.688

Note: BMI: body mass index; Gr: group; T: time; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; FM: fat mass; FFSTM: fat free soft tissue mass; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue. DXA-derived measures of body composition use subtotal (excluding the head) measures of FM and FFSTM. (#) DXA-derived body composition measures had missing data for one CTL participant at post-testing. * - $p<0.05$ post-hoc within-group change over time; $^{\varphi}$ - $p<0.05$ post hoc difference between groups at post-testing.

Cardiorespiratory Fitness, Metabolic Rate and Substrate Utilization

At baseline there were no group differences in VO_{2peak} whether unadjusted or adjusted for weight or FFSTM (p=0.774, p=0.291 and p=0.447 respectively)(Table 2.2). Baseline measures of EE, RER and rates of fat and carbohydrate oxidation (adjusted for FFSTM) were not different between EXE and CTL at rest or during steady state exercise (50% VO_{2peak}) (p>0.05). In response to exercise training, mean VO_{2peak} showed interaction effects (p<0.001), increasing in EXE (post hoc Δtime: p<0.001) and remaining unchanged in CTL (post hoc Δtime: p>0.05), when unadjusted or adjusted for weight or FFSTM. During steady state exercise, at post-testing EXE performed the same absolute workload at a lower $\%VO_{2peak}$ (interaction: 0.037; post hoc Δtime: p<0.001) and $\%HR_{peak}$ (p=0.001; post hoc Δtime: p<0.001). During steady state exercise at the same relative intensity (50% post-testing VO_{2peak}), there were interaction effects for EE ($kJ.FFSTM^{-1}$, p=0.006) which increased in EXE (post hoc Δtime: p<0.001) due to the higher workload required to reach this intensity (treadmill gradient: interaction: p<0.001; EXE post hoc Δtime, p<0.001). While there were no interaction effects for carbohydrate oxidation rates, there were for fat oxidation rates (p=0.009) such that the increased EE in EXE was fuelled by higher fat oxidation rates (post hoc Δtime: p=0.003). In contrast, rates of fat and carbohydrate oxidation remained unchanged in CTL (p>0.05). At rest there were no interaction effects for energy expenditure or substrate utilization between EXE and CTL.

Table 2.2: Cardiorespiratory fitness, energy expenditure and substrate utilization during submaximal exercise and at rest

	CTL (n=15)		EXE (n=20)		P value		
	Pre	Post	Pre	Post	Group x Time	Group	Time
Cardiorespiratory Fitness							
VO_{2peak} ($ml.min^{-1}$)	2099.4±282	2032.3±196	**2077±211**	**2278±231***	**0.001**	0.774	0.286
VO_{2peak} ($ml.min^{-1}.kg^{-1}$)	23.9±3.0	23.0±2.6	**24.9±2.4**	**27.6±3.4***	**<0.001**	0.291	0.195
VO_{2peak}($ml.min^{-1}.FFSTM^{-1}$)#	55.4±8.5	52.9±7.7	**57.1±5.6**	**62.5±5.3***	**<0.001**	0.447	0.144
Steady State (Absolute)							
VO_2 (%VO_{2peak})	52.3±3.7	51.3±8.1	**51.2±3.9**	**43.9±7.5***	**0.037**	**0.008**	**0.008**
HR (%HR_{peak})	65.4±5.5	64.8±5.5	**68.2±4.5**	**60.0±6.8***	**0.001**	0.146	0.643
EE ($kJ.h^{-1}.FFSTM^{-1}$)#	34.3±5.0	32.2±4.6	35.6±4.2	32.8±4.6	0.627	0.109	0.475
RER	0.847±0.042	0.850±0.032	0.848±0.048	0.824±0.044	0.069	0.924	0.759
Energy derived from Fat (%)	51.8±14.9	51.0±11.0	51.6±16.9	59.9±15.0	0.074	0.957	0.824
Fat oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)#	7.2±2.0	6.7±2.0	7.5±2.5	8.0±2.2	0.227	0.694	0.460
CHO oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)#	18.4±6.40	17.5±3.9	18.8±6.3	14.7±6.1	0.067	0.831	0.594
Steady State (Relative)							
VO_2 (%VO_{2peak})	52.3±3.7	52.7±3.7	51.2±3.9	51.3±3.2	0.892	0.342	0.710
HR (%HR_{peak})	65.4±5.5	65.7±5.5	68.2±4.5	65.5±5.0	0.126	0.107	0.831
EE ($kJ.h^{-1}.FFSTM^{-1}$)#	34.3±5.0	33.5±5.4	**35.6±4.2**	**38.9±4.2***	**0.006**	0.477	0.607
RER	0.847±0.042	0.857±0.027	0.848±0.048	0.835±0.041	0.116	0.921	0.353
Energy derived from Fat (%)	51.8±1.49	48.6±9.4	51.6±16.9	56.0±14.0	0.125	0.956	0.390
Fat oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)#	7.2±2.0	6.6±1.8	**7.5±2.5**	**9.0±2.7*** φ	**0.009**	0.207	0.344
CHO oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)#	18.4±6.4	19.0±4.6	18.8±6.3	18.7±5.1	0.683	0.827	0.652
Speed ($km.h^{-1}$)	3.0±0.1	3.0±0.0	3.0±0.1	3.0±0.0	0.833	0.766	0.266
Gradient (%)	4.0±3.1	4.3±2.3	**3.8±2.6**	**9.2±4.3***	**<0.001**	0.851	0.719
Resting Measurements							
RMR ($kJ.d^{-1}.FFSTM^{-1}$)#	150±19	161±20	145±28	156±28	0.930	0.569	0.093
RER	0.788±0.037	0.772±0.050	0.800±0.044	0.797±0.040	0.458	0.413	0.247
Energy derived from Fat (%)	71.8±12.9	77.1±16.4	68.1±15.5	68.7±14.0	0.469	0.453	0.283
Fat oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)#	**1.8±0.4**	**2.0±0.5***	1.7±0.5	1.8±0.5	0.429	0.523	**0.028**
CHO oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)#	1.9±1.0	1.6±0.9	2.1±0.9	2.2±1.3	0.411	0.547	0.377

HR: heart rate; HR_{peak}: peak heart rate; VO_{2peak}: peak volume of oxygen consumption; EE: energy expenditure; FFSTM: fat free soft tissue mass; RER: non-protein respiratory exchange ratio; RMR: resting metabolic rate. (#) DXA measurements of FFSTM were missing for one CTL participant at post-testing. For all measures that adjust for FFSTM at post-testing: CTL (n=14). * - $p<0.05$ post-hoc within-group change over time; φ - $p<0.05$ post hoc difference between groups at post-testing.

Dietary intake and Physical Activity

Table 2.3 details dietary energy and macronutrient intake and daily step count measured every 4 weeks over the 12-week intervention period in EXE and CTL. Energy intake remained unchanged in the EXE and CTL (kJ) and there were no interaction effects. There were interaction effects for carbohydrate (%TDEI: $p=0.014$) and fat (%TDEI: $p=0.040$). This reflected unchanged macronutrient intake for EXE throughout the intervention ($p>0.05$),

however CTL increased carbohydrate intake in week 12 compared to baseline (post hoc Δtime: p=0.045) and consequently consumed relatively more carbohydrate (post hoc group difference: p=0.019) and less fat (post hoc group difference: p=0.028) compared to EXE at week 12. Daily step count showed interaction effects for week 4, 8 and 12 compared to baseline (p=0.009, p=0.009, p=0.001 respectively) such that daily step count increased in EXE in weeks 4, 8 and 12 (post hoc Δtime: p<0.05), but did not change in CTL (p>0.05).

Table 2.3 – Energy Intake, Macronutrient Distribution and Daily Step Count

	CTL (n=15)	EXE (n=20)	Group x Time	Group	Time
EI (kJ)					
Baseline	8,138 (6,493-9,434)	8,369 (7,014-10,565)		0.481	
Week 4 [#]	7,489 (7,110-9,217)	7,838 (6,934-9,780)	0.759		0.278
Week 8	7,963 (6,306-8,921)	8,516 (7,363-9,659)	0.658		0.259
Week 12	8,429 (7,335-9,509)	8,443 (7,595-9,919)	0.519		0.939
Protein (%EI)					
Baseline	14.3±1.9	13.2±2.5		0.207	
Week 4[#]	13.2±2.3	13.9±2.9	0.108		0.204
Week 8	13.8±2.8	13.4±2.0	0.509		0.531
Week 12	13.5±2.1	13.7±3.0	0.278		0.408
Carbohydrate (%EI)					
Baseline	54.0±5.7	55.1±5.4		0.626	
Week 4[#]	56.9±6.0	53.9±5.9	0.098		0.121
Week 8	56.0±7.6	54.2±5.3	0.257		0.289
Week 12	**57.8±7.2***	**52.7±8.3**[φ]	**0.014**		**0.045**
Fat (%EI)					
Baseline	31.0±5.6	30.4±6.1		0.795	
Week 4[#]	29.2±5.1	31.0±6.0	0.389		0.202
Week 8	29.5±7.3	31.3±5.5	0.330		00424
Week 12	**27.8±6.4***	**32.2±5.9**[φ]	**0.040**		**0.082**
Daily step count					
Baseline	10,082±2,598	9,461±2,295		0.533	
Week 4[#]	**9,055±2,471**	**11,094±2,337***[φ]	**0.009**		**0.148**
Week 8	**8,784±2,785**	**10,661±3,517**[φ]	**0.009**		**0.067**
Week 12#	**9,242±1,791**	**11,627±3,505***[φ]	**0.001**		**0.190**

Note: EI: energy intake. * - p<0.05 post-hoc within-group change over time; [φ] - p<0.05 post-hoc differences between groups at baseline, 4week, 8 week or 12 week. Dietary intake data: #: at week 4, CTL (n=14). Daily step count: #: at week 4, EXE (n=19); at week 12, EXE (n=19), CTL (n=14).

Associations between EE and substrate utilization and body composition

Associations between baseline and changes in CRF and EE and substrate utilization at rest and during steady state exercise were explored in relation to changes in body composition in both EXE and CTL and presented in the Appendix (Supplementary Table 2.1). Higher baseline steady state exercise EE and fat oxidation rates (Figure 2.2A) were associated with

reductions in gynoid FM (kg) in EXE (r=-0.480 and r=-0.542, respectively p<0.05 for both) but not CTL (p>0.05). At rest in EXE only, lower baseline RER and carbohydrate oxidation rates (Figure 2.2B) were associated with reductions in FM (kg), gynoid FM (kg) and abdominal SAT (cm^2), but not VAT (cm^2), while higher baseline resting fat oxidation rates were associated with reductions in android FM (kg, p=0.037) and SAT (p=0.016). The relationship between the changes in gynoid FM with baseline VO_{2peak} (Figure 2.2C) and change in VO_{2peak} (Figure 2.2D) differed by group such that higher baseline VO_{2peak} and greater increases in VO_{2peak} were associated with a decrease in FM and gynoid FM in EXE (p<0.05) but not CTL (p>0.05).

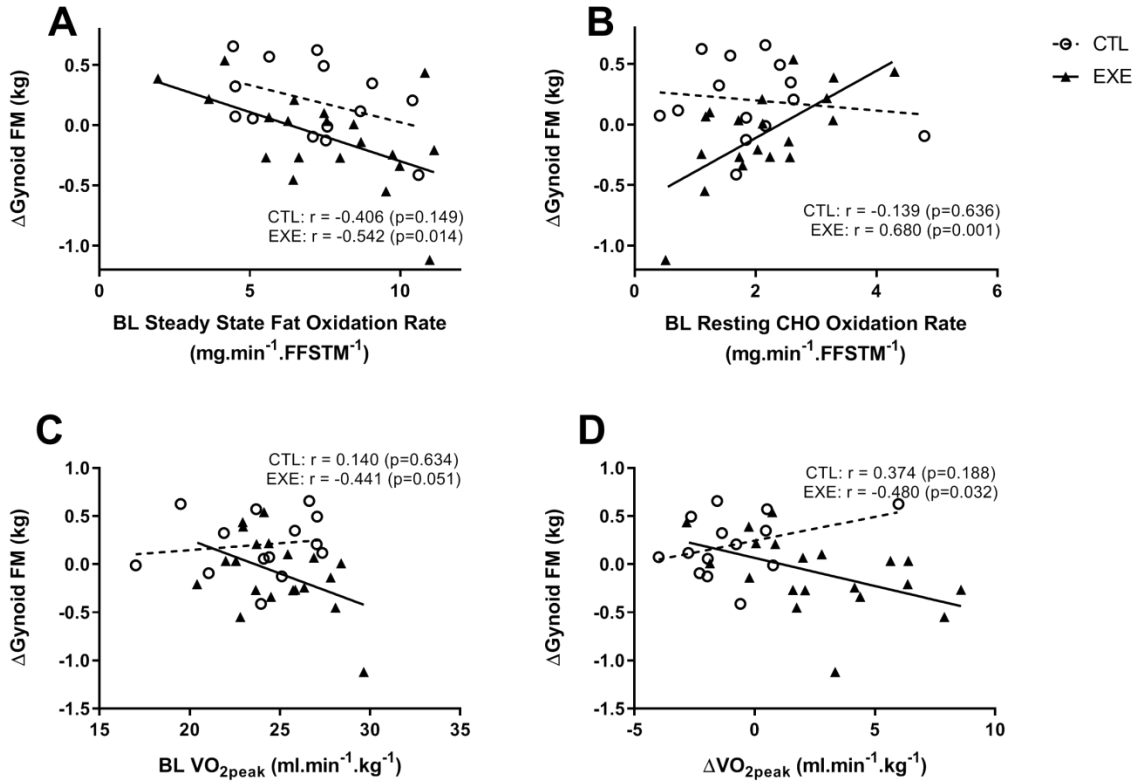

Figure 2.2: Associations between baseline substrate oxidation, baseline and change in CRF with changes in Gynoid FM in the exercise and control groups.

Note: FM: fat mass, FFSTM: fat free soft tissue mass, VO_{2peak}: peak volume of oxygen consumption, BL: baseline; CHO: carbohydrate.

Changes in EE and substrate utilization at rest and during exercise in response to exercise training showed that for EXE only, decreases in carbohydrate oxidation rates and RER at the same relative exercise intensity and at rest were associated with increases in gynoid FM (p<0.05) (Supplementary Table 2.1). Baseline values of carbohydrate oxidation rates and

RER were negatively associated with subsequent changes in response to exercise such that individuals with higher baseline carbohydrate oxidation rates and RER at rest (r = -0.588 & r = -0.750, p<0.001) and during steady state exercise (r = -0.669 & r = -0.704, p<0.001) showed the greatest reductions in response to exercise. However, individuals with a greater reliance on carbohydrate oxidation from the outset showed a more muted response with some showing increases, rather than reductions, in gynoid FM.

Table 2.4 below shows the regression models for predictors of change in gynoid FM in response to exercise training, and as such uses data for EXE only. Based on the bivariate associations presented in supplementary Table 1, significant independent variables were included to generate the most parsimonious model. In the model, baseline rates of resting carbohydrate oxidation and steady state fat oxidation explained over 60% of the variance in subsequent change in gynoid FM in response to the exercise intervention, with both measures being significant independent predictors. Including either baseline VO_{2peak} (R^2=0.625 (adj R^2=0.550), p=0.002) and ΔVO_{2peak} (R^2=0.642 (adj R^2=0.570), p=0.001) did not significantly improve the model and neither of these variables were independent predictors.

Table 2.4 – Predictors of change in gynoid FM (kg) in response to exercise

ΔGynoid FM	Coefficient	p-value	95% Confidence Interval	Beta Coefficient
Model: R^2=0.606 (adj R^2 = 0.557) p<0.001				
Baseline resting carbohydrate oxidation rate (mg.min^{-1} .FFSTM^{-1})	224,532	**0.004**	80,786 to 368,277	0.549
Baseline steady state fat oxidation rate (mg.min^{-1} .FFSTM^{-1})	-59,145	**0.028**	-111,036 to -7,254	-0.401

Note: FM: fat mass; FFSTM: fat-free soft tissue mass.

2.4 Discussion

The main finding of this study was that 12 weeks of combined aerobic and resistance exercise training in obese, black SA women resulted in an unanticipated relative reduction in gynoid FM rather than VAT, which is typically shown in response to exercise training[168]. Over 60% of the variance in change to gynoid FM in response to the exercise intervention was explained by baseline measures of fat oxidation during steady state exercise and carbohydrate oxidation at rest. These findings demonstrate that an increased ability to oxidize fat and reduced reliance on carbohydrate oxidation for energy metabolism at the outset facilitated fat mobilization in response to exercise training. As hypothesized, the exercise stimulus also

resulted in increased CRF from a very low base and improvements in EE and fat oxidation during steady state exercise, compared to no change in non-exercising controls.

This moderate to high-intensity exercise training intervention stimulated improvements in CRF and whole body fat oxidation rates during steady state exercise compared to controls who continued to engage in daily low intensity ambulatory activity. Improved CRF is not shown in all exercise intervention studies. In overweight and obese African American women, 14 weeks of high intensity interval training failed to achieve improvements in VO_{2peak}, suggesting that combined resistance and moderate-to-high intensity aerobic training may be preferable in achieving cardiorespiratory fitness (CRF) improvements[142]. Higher-intensity exercise training does result in increased maximal fat oxidation rates, as shown with higher-intensity track walking compared to lower-intensity, self-paced walking[139]. During steady state exercise at the same relative intensity (50% post-testing VO_{2peak}), EXE increased fat oxidation rates by ~20%. At the same absolute workload, while fat oxidation rates remained unchanged, carbohydrate oxidation rates tended to decline (p=0.067), consequently enabling EXE to increase the proportion of energy derived from fat from ~52% to ~60%. These results show that the exercise training stimulus was adequate to achieve improvements in CRF and fat oxidation rates during steady state exercise in this sedentary, obese population group. Reported dietary energy intake and macronutrient distribution remained unchanged in EXE so it is unlikely that this would have influenced changes in substrate utilization at rest or during steady state exercise.

Low CRF is found to be a determinant of insulin resistance, independent of physical activity[220]. The current study showed that baseline CRF levels for all participants was low, confirming previous findings[220]. Despite increasing CRF by 10.8% to 27.6±3.4ml.min^{-1}.kg^{-1} in response to the 12-week exercise training intervention, CRF remained slightly below the 20th percentile for VO_{2peak} previously shown in female African American populations (28.3 ml.min^{-1}. Kg^{-1}; 73% overweight or obese)[254]. This is of concern in a population group with a high prevalence of obesity (40.9%)[255] and metabolic syndrome (43%)[256]. These results highlight the importance of promoting similar exercise programs that are sustainable over the long term, to ensure ongoing improvements in CRF and related health risks within these communities[2,71–74].

In contrast to the exercise training response, metabolic rate and substrate utilization at rest remained unchanged in EXE following the intervention. Resting and steady state exercise testing was carried out in the fasted state, with dietary intake controlled on the night before, and activity controlled for 72 hours prior to the resting measurement. The lack of response in resting substrate utilization in the EXE, is consistent with results from other exercise training interventions in obese, sedentary populations of similar duration (12-14 weeks)[142,160] and longer (16 months), where no calorie restriction was applied[161]. However, decreases in resting RER are shown in shorter studies (7 weeks) where energy and carbohydrate intake were volitionally reduced [166] and in interventions incorporating calorie restricted weight loss of ~8%[167]. Nonetheless, skeletal muscle metabolism, a key target for exercise adaptation, contributes a significantly lower proportion of whole body substrate metabolism at rest in comparison to exercise. Therefore it is likely that resting substrate utilization is a less sensitive measure for assessing metabolic changes in response to exercise training, particularly in the absence of weight loss. By comparison, at post-testing, resting fat oxidation rates increased in CTL, which was unexpected given the increase in self-reported carbohydrate intake over the 12-week intervention period.

While exercising participants only achieved modest reductions in body weight, the control group had a small, but significant increase in body weight. The latter is in line with previous findings that showed large increases in body weight and central fat mass in young black SA women over a 5.5 year free-living period[219]. Hence, exercise training may be an effective intervention to attenuate gains in body weight and centralisation of body fat, and the associated reduction in insulin sensitivity in this population.

Notably, fat mobilization in the participants of this study occurred specifically in gynoid depots, and not VAT as is typically shown in overweight and obese adults in response to exercise training[168]. Although the absolute reduction in gynoid FM was relatively small, it is important to note that the gynoid depot as a proportion of total FM was significantly reduced in response to exercise training. This is a novel finding, potentially representing an ethnic-specific response to exercise training and warrants further investigation. Studies in SA and abroad have shown ethnic differences in body fat distribution between black African and white women. In particular, compared to their white counterparts, black women have less central and more peripheral, lower-body FM and have less abdominal VAT and more SAT for a given WC[85,237,257]. In comparison to white women, the gluteal fat depot of black SA

women shows differential gene expression characterised by a higher inflammatory profile compared to abdominal SAT depots[89], a reduced adipogenic and lipogenic profile[90], greater adipocyte hypertrophy[87,88], reduced vascularization[88,89] and increased hypoxia[90]. All of these factors are implicated with ectopic fat deposition and further increases in insulin resistance[74], therefore reductions in gynoid FM in response to exercise training may help to attenuate this risk.

Among exercise participants, 60% of the variability in changes to gynoid FM in response to exercise training was explained by baseline rates of fat oxidation during steady state exercise and carbohydrate oxidation at rest. This suggests that higher fat oxidative capacity is important for achieving body composition changes in response to exercise training and supports previous findings[166,258,259]. Unfortunately, as recently highlighted[260], substrate utilization during submaximal exercise is not routinely measured in exercise intervention studies. Equally the relationship between substrate oxidation rates and subsequent body composition changes are not commonly explored, despite high inter-individual variability in body composition response to exercise training[142,234]. Accordingly, the current study suggests that higher fat oxidation during steady state exercise in this population group may increase fat mobilisation particularly in the gluteal depot in response to exercise training.

This is the first study to investigate changes in substrate oxidation at rest and during steady state exercise in response to 12 weeks of exercise training in obese black SA women, a population group at increased risk for obesity and T2DM. It builds on the body of evidence supporting the role of fat oxidation in achieving body composition changes in response to exercise training, independent of changes in diet. Notably, the study shows that exercise training results in mobilization of gynoid FM in this sample of obese black SA women, which is a novel finding that is in contrast to previous exercise interventions. By design this study was aimed at evaluating an exercise intervention in black SA women, as such, the study did not allow for comparison with other ethnic groups, but it would certainly be of interest to explore the differential response of body composition changes to exercise training between different ethnic groups and sexes. Although not representing free-living conditions, the assessment of submaximal exercise under fasted conditions allowed the underlying changes in substrate utilization, independent of the acute influence of dietary intake, to be examined. Resting measurements were undertaken at least 72 h after the last exercise session and food intake 12 h prior to testing was standardized and monitored. The exercise intervention was

supervised and monitored throughout by a trained human-movement and exercise specialist and objectively monitored using heart rate monitoring to ensure adherence to the required exercise intensity. In free living conditions it is recognised that supervised exercise training such as this requires financial support and as such would be intended for individuals who had access to similar programs, either privately funded or supported through community-based government initiatives. While it is recognised that self-reported dietary data collection is inherently problematic, 24 h recalls were completed with a registered dietitian who also reviewed the self-reported intake to ensure record completeness. However, it is noted that using comparisons of reported EI relative to recorded RMR under reporting, as defined by Goldberg et al (1991) cut-off of 1.35 (Supplementary table 3.3), was evident[261]. The sample size was also relatively small and findings may have been strengthened by increased numbers.

In conclusion, exercise training in sedentary, obese black SA women improved CRF and fat oxidation rates during submaximal exercise. Notably, higher fat oxidation rates during steady state exercise and lower resting carbohydrate oxidation at baseline, together with improvements in CRF, were associated with the mobilization of gynoid FM, rather than VAT as hypothesized, in response to exercise training. This relationship may help to explain inter-individual variability in body composition responses to exercise interventions. Similar exercise training programs, that are sustainable over the long term, would therefore be beneficial in achieving meaningful improvements in CRF and associated risk factors while also supporting weight management and body composition improvements in this high risk population group.

CHAPTER 3

3. Higher carbohydrate oxidation rates at rest and during exercise predict poorer VO_{2peak} response to an exercise intervention in sedentary, obese black South African women

Louise Clamp

For this study I was responsible for: developing the research questions; advertisement and recruitment of participants; carrying out the graded exercise test to determine VO_{2peak}; carrying out the steady state exercise testing; carrying out the resting measurements; collection and analysis of dietary data; statistical analysis and interpretation and write-up of the manuscript.

3.1 Introduction

Low levels of physical activity, and in particular low CRF, are strongly associated with increased markers of cardiovascular disease and T2DM, with low CRF independently predicting mortality in asymptomatic women[25,224,225,262]. VO_{2peak} is widely used to measure CRF and to assess the effectiveness of exercise interventions in improving CRF[144]. However, high inter-individual variability in CRF response to exercise interventions, beyond that observed in non-exercising controls, has been documented and is frequently attributed to differences in the exercise dose (volume and intensity)[140,144–148]. While exercise dose undoubtedly plays a role in the outcome, underlying variability in metabolic and physiological variables at the outset, particularly among sedentary individuals, may also contribute to the subsequent variability in CRF response to exercise training.

Exercise interventions can improve CRF, fat oxidative capacity, body composition and S_I[112,142,167,176,263,264]. CRF, specifically improved through exercise training, is highly correlated with higher fat oxidation rates during exercise and differs between well trained and untrained individuals[153,265]. Furthermore, low rates of fat oxidation and a preference for carbohydrate oxidation has been shown to increase short term ad libitum food intake and weight gain[60] and predict longer term increases in body weight, obesity and FM[63,65,66,154,266]. Conversely, exercise training increases fat oxidation at rest and during submaximal exercise, potentially supporting fat mobilization and body composition improvements[55,142,166,267,268]. Despite this, the relationship between fat oxidative capacity and subsequent body composition changes in response to exercise interventions are not commonly explored, despite evidence of high inter-individual variability in body composition responses to the interventions[142,234]. Exercise training, potentially through increased skeletal muscle oxidative capacity [122], also improves insulin sensitivity[167,176,269]. It is therefore of importance to explore the role of substrate utilization in determining the CRF response to exercise training, as well as the impact it may have on secondary outcomes such as improving body composition and insulin sensitivity.

Within SA, black women have a very high prevalence of obesity and T2DM [85,221,222,270]. We and others have shown that black SA women have very low levels of CRF, well below the 20th percentile previously shown in African American women[271] and below the 5th percentile for age and gender norms[220,241]. However, in the previous chapter I showed that 12 weeks of

exercise training in obese black SA women resulted in improved VO_{2peak} and a reduction in body weight compared to non-exercising controls. I hypothesized that there would be high inter-individual variability in CRF response to the exercise intervention, and that there would be underlying differences in substrate utilization at baseline between participants with low and high CRF response to the exercise intervention. I further hypothesized that these differences would lead to differential changes in body composition and insulin sensitivity in response to the exercise intervention.

The aims of this study were therefore to: 1) examine the variability in ΔVO_{2peak}, as a proxy of changes in CRF, in response to the 12-week exercise intervention; 2) compare changes in EE and substrate utilization at rest and during steady state exercise in response to the 12 week exercise intervention between high and low VO_{2peak} responders; and 3) compare changes in body composition and insulin sensitivity in response to the 12 week exercise intervention between high and low VO_{2peak} responders. Lastly, I aimed to identify whether differences in baseline substrate utilization, at rest and during exercise, may explain the variability in CRF response to exercise training.

3.2 Methods

Study design

All research protocol procedures and baseline characteristics of the participants have been described previously[272]. This chapter uses data from the same obese black SA female participants (exercise group: EXE; n = 20; control group: CTL; n = 15) of the 12 week exercise intervention study, collected as described in the previous chapter. In summary, participants underwent 3 pre- and post-intervention testing sessions. At the first session body weight, anthropometry and body composition (DXA) measurements were taken; thereafter participants completed a graded exercise test to determine VO_{2peak} and HR_{peak}. At the second session a steady state exercise test was carried out to determine EE and substrate utilization at 50% VO_{2peak}. At post-testing, two steady state exercise tests were carried out with a 10 minute rest in between. The first was conducted at the same *absolute* workload (treadmill speed and gradient) and the second at the same *relative* exercise intensity (50% of post-testing VO_{2peak}). Over 72h thereafter, participants consumed a standardized evening meal and fasted overnight (10-12h), remaining at the facility. At 6am the next morning, resting metabolic rate (RMR) and substrate utilization was measured and a frequently sampled

intravenous glucose tolerance test (FSIGT) was completed to determine insulin sensitivity index (S_I)(see description below). Following pre-intervention testing, EXE then completed 12 weeks of supervised aerobic and resistance training at moderate-vigorous intensity (>75% peak heart rate, HR_{peak}), progressing from 40 to 60 min per session, 4 days per week for 12 weeks. Heart rate monitors (Polar A300, Kempele, Finland) were worn at all exercise sessions to ensure adherence to the prescribed exercise intensity. CTL were instructed to continue with habitual diet and physical activity patterns. Dietary intake (one 24h recall and 3-d food records to include one weekend day) and physical activity data over 7 days (ActivPAL, activPAL3c, PAL Technologies Ltd, Glasgow, UK) were collected at pre-testing, 4, 8 and 12 weeks.

Insulin sensitivity

Fasting blood samples were taken after completion of the RMR measures. Glucose (50% dextrose; 11.4 g/m^2 body surface area) was intravenously infused over 60 s starting at time 0. Human insulin (0.02 U/kg; NovoRapid, Novo Nordisk, Bagsvaerd, Denmark) was infused at a constant rate over 5 min (starting at t=20min) (HK400 Hawkmed Syringe Pump, Shenzhen Hawk Medical Instrument Co., Shenzhen, China). Blood samples were then taken at time intervals as stated in the protocol[241] and were analysed to determine plasma glucose and insulin concentrations. Plasma glucose concentrations were determined using a colorimetric assay (Randox, Gauteng, South Africa) and serum insulin was measured using immunochemiluminometric assays (IMMULITE 1000 immunoassay system, Siemens Healthcare, Midrand, South Africa). Bergman's minimal model of glucose kinetics [273] was used to calculate the S_I from glucose and insulin concentrations obtained during the FSIGT[241].

Cardiorespiratory fitness response: high and low responders

CRF response to exercise training was defined as the change in relative VO_{2peak} (per kilogram body mass), determined from the graded exercise test, from pre- to post-testing. The true inter-individual response was quantified by the following formula proposed by Williamson et.al., 2017[274]:

$$\boldsymbol{SD_R = \sqrt{(SD^2_I - SD^2_C)}}$$

where SD_R is the true inter-individual response and SD_I and SD_C is the standard deviation (SD) of the change in VO_{2peak} in the EXE (I) and CTL (C) groups respectively. Based on

gender and age associated norms (females, 18-30yrs 41-44ml.min^{-1}.kg^{-1})[271,275], true inter-individual variability of relative VO_{2peak} of 8-8.5% (~1 MET = 3.5ml.min^{-1}.kg^{-1}) would be considered clinically meaningful[274,276]. High responders (HRS) were identified as those achieving above median change in $VO_{2peak,}$ and low responders (LRS) were identified as those achieving below median changes in VO_{2peak} . HRS and LRS were compared with respect to changes in primary and secondary outcome variables.

Statistical Analysis

Statistical analysis was carried out using Stata (Version 12, StatCorp, College Station, Texas 77845, USA). Data was assessed for normality using the Shapiro Wilks test. Mean and standard deviations were reported for normally distributed data and median and inter-quartile-ranges for non-parametric data. Comparisons of single variables (e.g., number of classes attended, average exercise intensity) of normally distributed data used two sample t-tests for independent groups with equal variance, or a Satterthwaite's independent sample t-test for unequal variance. Wilcoxon Rank Sum test was used for non-parametric data. Mixed models with repeated measures were used to compare differences in the changes in outcome variables between groups in response to the intervention. Post-hoc tests determined differences between groups at baseline or post-testing as well as within-group changes over time. Pearson and Spearman correlation coefficients were used to examine bivariate associations and multiple linear regression models were used to model predictors of change in VO_{2peak}. The multiple linear regression models were tested for normality of residuals, linearity and homoscedasticity. Outliers were checked for influence and leverage and multicollinearity of predictors was assessed using the variance inflation factor (VIF > 5). For all tests, significance was accepted at $p<0.05$.

The analysis of CRF response variability excluded the control group not exposed to exercise training and focused primarily on the exercise group, comparing high and low responder groups with respect to changes in primary and secondary outcome variables. Comparison of LRS and HRS to CTL is however also provided with results shown in Supplementary Tables.

3.3 Results

Inter-individual variability in CRF response: exercise versus control groups

VO_{2peak} was unchanged in the CTL (ΔVO_{2peak}: -0.92 ±2.32ml.min^{-1}.kg^{-1}, p=0.195) and increased in the EXE ((ΔVO_{2peak}: +2.67 ±3.15ml.min^{-1}.kg^{-1}, p<0.001; group-by-time interaction: p<0.001)(Figure 3.1, below). True inter-individual response variability for the EXE was calculated to be ±8.4%, after accounting for the variability observed in the non-exercising control group. Based on a median split, high responders (HRS, n=10) and low responders (LRS, n=10) were identified as those achieving above or below median changes in VO_{2peak}. HRS increased VO_{2peak} by 21.7±10.0% (p<0.001) while in LRS this remained unchanged (+0.6±6.3%, p=0.748, group-by-time interaction: p<0.001) (Table 3.1).

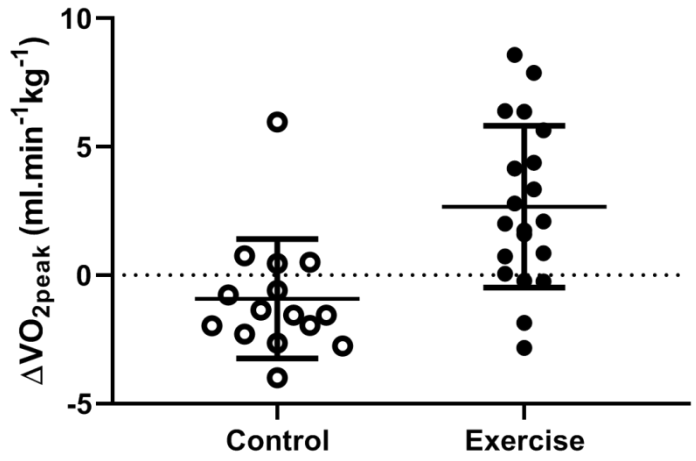

Figure 3.1: Variability in the change in cardiorespiratory fitness in exercise participants compared to non-exercising controls.

Note: $VO_{2peak:}$ peak volume of oxygen consumption.

Baseline characteristics, exercise and resting tests – Low and High Responders

The characteristics of the LRS and HRS groups are described in Table 3.1, with the additional comparison with the control group presented in the Appendix (Supplementary Table 3.1). There was no difference in age (p=0.249) between LRS and HRS. At baseline, absolute VO_{2peak} was higher in LRS than HRS (p<0.001), but when adjusted for body weight or FFSTM, baseline VO_{2peak} was not different between groups (p>0.05). Both groups performed the baseline steady state exercise test (50% VO_{2peak}) at a similar workload (treadmill speed and gradient), although RER (p<0.001) and carbohydrate oxidation rates

were higher ($p<0.001$), and fat oxidation rates were lower ($p=0.005$) in LRS compared to HRS (Figure 3.2). At rest, there were no group differences in baseline RMR, RER, fat or carbohydrate oxidation rates ($p>0.05$) (Figure 3.2). LRS were however 10.8 kg heavier ($p=0.001$) at baseline and had greater BMI ($p<0.001$), HC ($p=0.005$), FM ($p=0.027$), FFSTM ($p=0.006$) and SAT ($p=0.010$) compared to HRS. By contrast, VAT, BF%, WHR and proportional android and gynoid FM (%FM) were not different between LRS and HRS ($p>0.05$). Baseline fasting plasma glucose, insulin and S_I were also similar between LRS and HRS ($p>0.05$).

Table 3.1: Age, exercise adherence, CRF, Steady state test work rate and workload, body composition and insulin sensitivity for high and low VO_{2peak} responders to a 12 week exercise intervention

	LRS (n=10)		HRS (n=10)	
Age (yrs)[A]	22(21-24)		22.5(21-27)	
Exercise Adherence:				
Classes attended[B]	38.2±6.0		38.0±6.9	
HR (%HR_{peak})[B]	78.6±4.0		80.6±4.0	
	Baseline	**Post-testing**	**Baseline**	**Post-testing**
Cardiorespiratory Fitness:				
VO_{2peak} ($ml.min^{-1}$)	**2,233±100**	2,242±199	**1,921±173****	**2,314±264***
VO_{2peak} ($ml.min^{-1}.kg^{-1}$)	25.3±2.2	**25.5±2.9**	**24.5±2.7**	**29.7±2.5* ****
VO_{2peak} ($ml.min^{-1}.FFSTM^{-1}$)	58.4±3.5	**58.7±3.4**	**55.8±7.2**	**66.3±4.0* ****
Steady State (Absolute):				
VO_2 (%peak)	50.6±3.1	**47.9±3.1**	**51.7±3.1**	**39.9±3.2* ****
HR (%HRpeak)	**68.7±3.5**	**62.5±7.0***	**67.6±5.5**	**57.5±6.0* ****
Speed ($km.h^{-1}$)	3±0	3±0	3±0	3±0
Gradient (%)	4.0±2.5	4.0±2.5	3.6±2.8	3.6±2.8
Steady State (Relative):				
VO_2 (%peak)	50.6±3.1	51.7±2.1	51.7±3.1	51.3±2.1
HR (%HRpeak)	68.7±3.5	65.7±6.1	67.6±5.5	65.4±4.0
Speed ($km.h^{-1}$)	3±0	3±0	3±0	3±0
Gradient (%)	**4.0±2.5**	**7.8±4.0***	**3.6±2.8**	**10.6±4.2***
***Body Composition*:**				
Weight (kg)	**89.5±6.0**	**88.4±7.8**	**78.7±7.7****	**78.2±8.9****
Height (m)	157.7±3.5		156.2±7.7	
BMI ($kg.m^{-2}$)	**36.2**	**35.9**	**31.8****	**31.0****
	(35.3-37.1)	**(34.8-37.5)**	**(30.6-33.9)**	**(30.0-34.5)**
WC (cm)	**108**	**102***	**100**	**97* ****
	(100-109)	**(95-109)**	**(98-105)**	**(94-103)**
HC (cm)	**118**	**117***	**113**	**110* ****
	(114-123)	**(112-123)**	**(105-116)**	**(104-113)**
WHR	0.89	0.87	0.90	0.89
	(0.87-0.93)	(0.86-0.90)	(0.87-0.94)	(0.86-0.91)
FFSTM (kg)	**39.0**	**39.3**	**34.3****	**35.3****
	(36.8-40.3)	**(35.9-40.1)**	**(32.7-37.4)**	**(33.0-37.2)**
BF%	49.9	50.2	50.0	48.8
	(48.7-51.7)	(49.3-52.8)	(48.3-51.6)	(48.1-50.4)
FM (kg)	**41.2±5.1**	**42.0±6.5**	**36.1±4.9****	**35.3±5.3****
Android FM (%FM)	8.2±.8	8.1±0.9	8.3±1.3	8.2±1.2
Gynoid FM (%FM)	18.7±1.8	18.6±1.5	**18.2±1.7**	**17.8±1.6***
SAT (cm^2)	**566±75**	**565±81**	**491±54****	**482±60****
VAT (cm^2)	131.1	140.9	125.2	129.1
	(121.9-165.0)	(119.1-152.4)	(121.9-163.8)	(121.6-136.3)
Insulin Sensitivity[#]:				
Fasting glucose ($mmol.L^{-1}$)	5.6 ±1.0	5.0 ±.0	5.4 ±0.7	5.2 ±1.0
Fasting insulin ($mU.L^{-1}$)	15.5	13.0	12.2	11.2
	(14.2-19.0)	(12.3-16.3)	(6.4-19.9)	(10.5-17.1)
S_I	**2.1**	**2.3 ***	1.7	2.0
	(1.3-3.1)	**(1.5-4.7)**	(1.2-2.4)	(1.5-3.1)

Note: HR: heart rate; HR_{peak}: peak heart rate; VO_{2peak}: peak volume of oxygen consumption; BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; FFSTM: fat free soft tissue mass; BF%: body fat percentage; FM: fat mass; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; S_I: insulin sensitivity index. * - post-hoc within group change over time ($p<0.05$); ** - post hoc group difference at pre-testing or post-testing ($p<0.05$). Superscript A: Wilcoxon's rank sum; superscript B: two sample t-test for independent groups with equal variance. #: All values for insulin sensitivity for pre- and post-testing, HRS (n=9).

When comparing the groups to the CTL (Supplementary Table 3.2), body composition did not differ between CTL and LRS at baseline, but CTL were heavier (p=0.013), with greater FM (p=0.043), FFM (p=0.032) and hip circumference (p=0.002) than HRS. Baseline VO_{2peak}, expressed per kg body weight, was not different to LRS (p=0.182) or HRS (p=0.547, Supplementary Table 3.1). During baseline steady-state exercise, RER was lower in CTL than LRS (p=0.036), but higher than in HRS (p=0.056). CTL also had lower carbohydrate oxidation rates compared to LRS (0.023). There were no differences in either RMR or resting substrate utilization measures or in fasting glucose, insulin and S_I between CTL, LRS and HRS at baseline (p>0.05).

Exercise adherence and change in steady-state exercise intensity and workload in response to the 12-week exercise intervention

Despite marked differences in VO_{2peak} response to exercise training, both HRS and LRS showed no difference in exercise class attendance (p=0.946) and exercised at a similar average exercise intensity throughout the 12-week intervention ($\%HR_{peak}$, p=0.287) (Table 3.1). Due to improved VO_{2peak}, HRS performed the same absolute steady-state workload at a lower intensity at post-testing (39.9±3.2% VO_{2peak}, p<0.001), while LRS showed no change (p=0.233). At the same relative intensity, both LRS (+3.8 ±2.9%) and HRS (+7.0 ±2.4%) increased workload tolerance, indicated by greater treadmill gradient at post-testing (p<0.001 for both).

Compared to both LRS and HRS, VO_{2peak} remained unchanged in CTL (pre: 23.9±3.0 and post: 23.0±2.6ml.min^{-1}.kg^{-1}, p=0.075) and was lower than both LRS (p=0.017) and HRS (p<0.001) at post-testing (Supplementary Table 3.1). CTL performed the same absolute steady state workload at the same intensity at post-testing (51.3±8.1% VO_{2peak} p=0.613) which was similar to LRS (p=0.135) but higher than HRS (p<0.001). At the same relative steady state intensity, CTL did not increase workload tolerance and exercised at a similar treadmill gradient which was lower than both LRS (p=0.004) and HRS (p<0.001) at post-testing.

Change in EE and substrate utilization during steady state exercise in response to the 12 week intervention

The change in RER, EE and rates of fat and carbohydrate oxidation in response to the 12 week exercise intervention, during both steady state exercise (absolute and relative) and at rest are presented in Figure 3.2. At the same absolute workload, although RER tended to decline in LRS (p=0.055) in response to the exercise intervention, it remained higher than that of HRS (p=0.001) at post-testing. Accordingly, while carbohydrate oxidation, measured at the same absolute exercise workload rates, decreased in both groups (LRS: p=0.008; HRS p=0.031) in response to the exercise intervention, post-testing carbohydrate oxidation rates remained higher (p<0.001) while fat oxidation rates remained lower (p=0.037) in LRS compared to HRS. At the same relative exercise intensity (50% post-test VO_{2peak}), increased VO_{2peak} and greater workload tolerance in HRS translated into higher EE (p<0.001), which was fuelled by higher fat oxidation rates (p=0.047). By comparison, given the minimal CRF response in the LRS group, EE at 50% VO_{2peak} remained unchanged (p=0.568). Nonetheless, RER tended to decline in LRS (p=0.059), reflecting tendencies for lower carbohydrate oxidation rates (p=0.056) and higher fat oxidation rates (p=0.081).

While substrate utilization in CTL at the same absolute steady state workload was not different to LRS (p>0.05) at post–testing, RER (<0.001) and carbohydrate oxidation rates (p=0.001) were higher than in HRS, while fat oxidation rates tended to be lower (p=0.055) (Supplementary Table 3.1). During relative steady-state exercise, at post-testing RER and carbohydrate oxidation rates in LRS were no longer higher than those in CTL. However, compared to HRS, RER (p=0.009) remained higher and fat oxidation rates (p<0.001) were lower at the same relative intensity in CTL.

Change in RMR and substrate utilization at rest

While RMR was not different between groups (Figure 3.2D, interaction, p=0.362), resting RER declined in LRS (p=0.024) and increased in HRS (p=0.052) in response to the 12-week intervention (Figure 3.2B). There were concomitant interaction effects for rates of fat (Figure 3.2F, p=0.055) and carbohydrate oxidation (Figure 3.2H, p=0.014) such that fat oxidation rates increased in LRS (post hoc Δtime: p=0.029), while carbohydrate oxidation rates increased in HRS (post hoc Δtime: p=0.058) only. Resting EE and substrate utilization were unchanged in CTL, but at post-testing RER (p=0.006) and carbohydrate oxidation rates

(p=0.012) were lower, while fat oxidation rates (p=0.019) were higher in CTL compared to HRS (Supplementary Table 3.1).

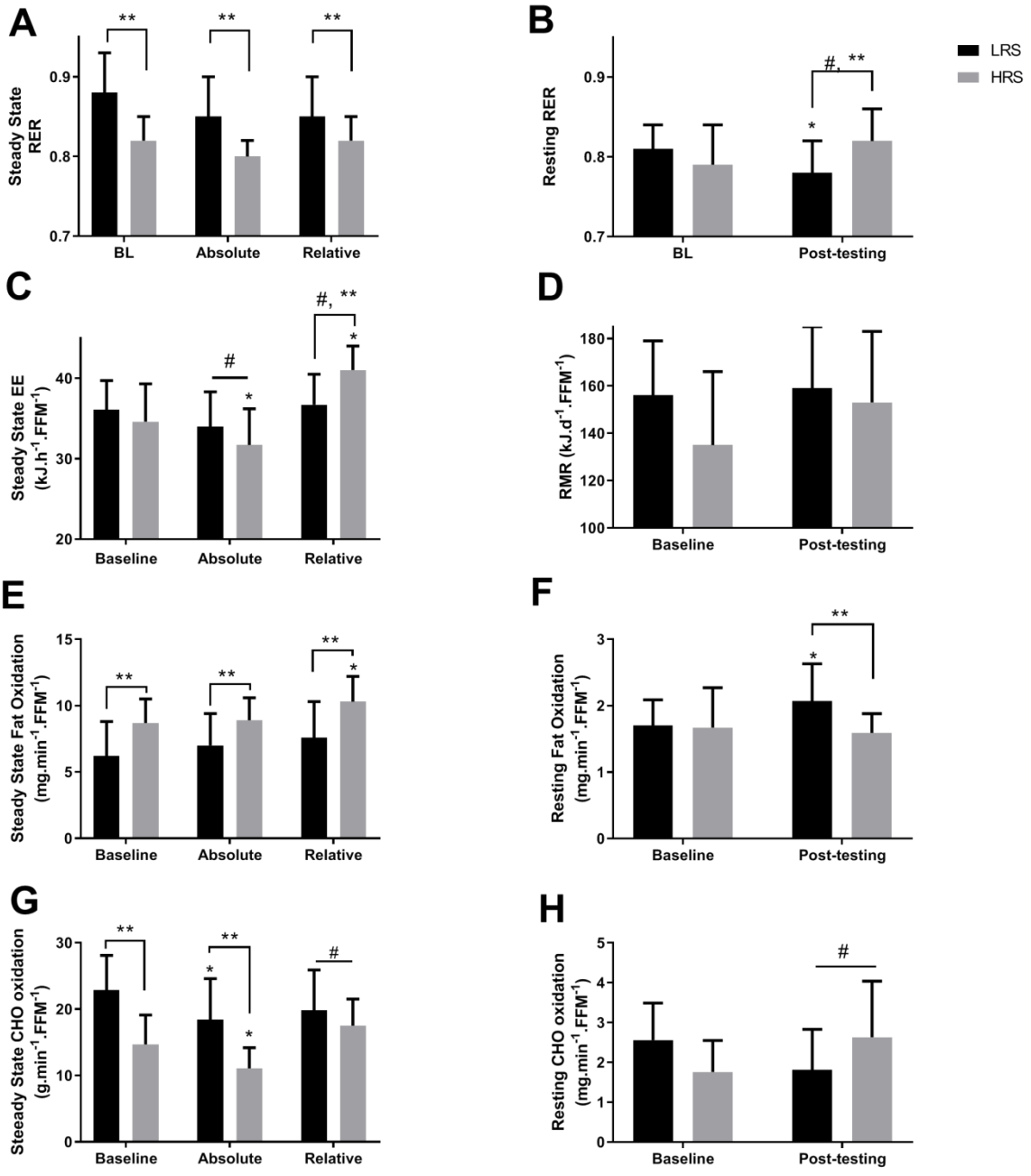

Figure 3.2: Changes in substrate utilization and energy expenditure during steady state exercise and at rest in response to the 12-week exercise intervention.

Values shown are mean and standard deviations. Note: Absolute Steady State Testing: 50% baseline VO_{2peak}; Relative steady state testing: 50% post-testing VO_{2peak}. BL: baseline; EE: energy expenditure; CHO: carbohydrate oxidation rate; RMR: resting metabolic rate; RER: respiratory exchange ratio. # - group-by-time effect ($p<0.05$); * - post-hoc change over time ($p<0.05$); ** - post hoc group difference at pre-testing or post-testing ($p<0.05$).

Change in body weight and composition in response to exercise intervention:

Body weight and BMI did not change significantly over the 12 week intervention in either group (within group change over time, p=0.132 and 0.126 respectively) and consequently the weight and BMI differential present at baseline between the two groups remained present at post-testing (p=0.002 and p<0.001 respectively)(see Table 3.1). WC decreased in both groups in response to the exercise intervention (HRS p<0.001; LRS p=0.013), although at post-testing WC was lower in HRS than LRS (p=0.039). HC reduced only in HRS (p=0.047), but remained unchanged in LRS. FM (kg) showed a tendency for an interaction effect such that FM decreased in HRS and increased in LRS (group-by-time, p=0.056), but this interaction effect was not observed for percentage body fat (group-by-time, p=0.143). There were no differences in the change in android FM (%FM), SAT (cm^2) or VAT (cm^2) between the two groups in response to the exercise intervention. By contrast gynoid FM was reduced in HRS (%FM, p<0.001; kg p=0.005) and remained unchanged in LRS (%FM p=0.272 and kg p=0.317), in response to the exercise intervention (group-by-time interaction: %FM p=0.040 & kg p=0.008 respectively).

Body weight (p=0.030), BMI (p=0.038) and WC (p=0.015) increased in CTL and there were group-by-time effects compared to both LRS (p=0.006, p=0.007 and p=0.001 respectively) and HRS (p=0.022, p=0.020 and p<0.001 respectively), where body weight and BMI remained relatively unchanged while WC decreased (Supplementary Table 3.2). The increase in FM in CTL approached significance (p=0.060) with a group-by-time effect for the comparison with HRS (p=0.021). There was also an interaction effect for the change in gynoid fat mass (% FM) which increased in CTL compared to a decrease in HRS (group-by-time: p<0.001).

Change in insulin sensitivity

Median fasting plasma glucose concentrations were within the normal range in both LRS and HRS at baseline and post-testing and did not change in response to the exercise intervention (Table 3.1). Similarly, fasting insulin concentrations did not differ between groups or in response to the exercise intervention (p>0.05). In contrast, the increase in S_I in response to exercise training was only significant for LRS (p<0.001). By comparison fasting glucose (p=0.827), fasting insulin (p=0.968) and S_I (p=0.265) were unchanged in CTL over the 12 weeks (Supplementary Table 3.2) and there was a group-by-time effect for S_I between LRS

and CTL (p=0.012) such that S_I improved in LRS (p=0.018) while remaining unchanged in CTL (p=0.265).

Changes in dietary Intake & Physical Activity in the HRS and LRS groups over the 12-week exercise intervention:

The dietary energy and macronutrient intake and daily step count at pre-testing, 4, 8 and 12 weeks are presented in Table 3.2. There were no differences in energy intake or macronutrient distribution of dietary intake between exercise groups at baseline or in response to the exercise intervention and dietary intake did not change over time for either group (p>0.05). By contrast CTL increased their carbohydrate intake (expressed as a % EI) in week 12 compared to baseline and consumed a greater proportion of carbohydrate than the HRS at week 12 (p=0.037) (Supplementary table 3.3). Supplementary table 3.3 also shows a comparison of reported EI:RMR at pre-= and post-testing. The degree of under reporting of dietary energy intake as defined by Goldberg et al (1991; EI:RMR < 1.35)[261] was 40% at pre-testing (CTL and LRS) and post-testing (all 3 groups). There were no differences in average daily step count between HRS and LRS at any time point, although both groups increased daily step count from baseline to week 12 (p=0.006). In contrast CTL reduced their daily step count over the 12 weeks and there were group-by-time effects with both exercise groups at week 4 (LRS: p=0.036, HRS: p=0.023), 8 (LRS: p=0.036, HRS: p=0.023) and 12 (LRS: p=0.036, HRS: p=0.023).

Table 3.2: Dietary Energy and Macronutrient Intake & Physical Activity at baseline, 4, 8 and 12 weeks for Low and High responders to a 12 week exercise intervention

	LRS	HRS
Dietary intake:		
Energy Intake (kJ)		
Baseline	9,633±4,118	8,602±2,075
Week 4	7,786±2,210	8,479±1,911
Week 8	9,008±2,827	8,522±1,376
Week 12	8,572±1,752	8,657±1,673
Protein (%EI)		
Baseline	13.2±2.8	13.3±2.4
Week 4	14.0±3.0	13.8±3.0
Week 8	13.4±1.9	13.4±2.2
Week 12	14.3±3.6	13.2±2.5
Carbohydrate (%EI)		
Baseline	55.5±6.5	54.6±4.5
Week 4	55.6±5.1	52.2±6.4
Week 8	55.4±5.0	53.1±5.6
Week 12	53.0±6.4	52.4±10.2
Fat (%EI)		
Baseline	30.3±7.8	30.6±4.0
Week 4	29.5±5.5	32.5±6.4
Week 8	30.2±5.2	32.4±5.8
Week 12	32.1±3.7	32.3±7.7
Physical activity		
Daily step count		
Baseline	9,118±2,688	9,843±1,846
Week 4[#]	10,436±2,498	11,827±2,031
Week 8	10,368±3,104	10,955±4,035
Week 12[#]	**11,666±3,052***	**11,587±4,077***

Note: EI: energy intake. *p<0.05 for within-group change over time; **p<0.05 for differences between groups at baseline, 4 week, 8 week or 12 week. Daily step count: #: week 4: HRS (n=9); week 12: HRS (n=9).

Associations between CRF response and exercise adherence, baseline substrate metabolism (at rest and during exercise), body composition and insulin sensitivity and dietary intake and physical activity during the intervention

When exploring bivariate associations in response to the exercise intervention, there were no associations between ΔVO_{2peak} and either the number of exercise sessions attended or the average exercise intensity of the training sessions completed. Furthermore, baseline relative VO_{2peak} was not associated with ΔVO_{2peak} in response to the exercise intervention ($p>0.05$). However, greater increase in ΔVO_{2peak} was associated with lower baseline carbohydrate oxidation rates during steady state exercise ($r=-0.462$, $p=0.040$) with a tendency for an

association at rest (r =-0.447, p=0.055). Higher ΔVO_{2peak} was associated with lower baseline BMI (r =-0.477, p=0.033) and SAT (r=-0.450, p=0.046), while lower baseline HC (r = -0.438, p=0.053) approached significance. Dietary variables and daily step count were not related to ΔVO_{2peak}. The linear regression model (Table 3.3) showed that baseline carbohydrate oxidation rates during steady-state exercise and at rest explained 37.5% (p=0.023) of the variability in CRF response to exercise training. When SAT was added to the model it explained 42.2% (p=0.037) of the variability in CRF response with only baseline carbohydrate oxidation during steady state exercise approaching significance as an independent predictor (p=0.065). The change in S_I was positively associated with RER (r = 0.448, p< 0.100) and carbohydrate oxidation rates (r= 0.431, p< 0.100) during steady state exercise at baseline, but was not associated with changes in CRF, EE or substrate utilization at rest or during exercise.

Table 3.3: Association between ΔVO_{2peak} and baseline measures of metabolic rate and substrate utilization at rest and during steady state exercise and body composition

	ΔVO_{2peak}			ΔVO_{2peak}	
	Correlation Coefficient (r)	p-value		Correlation Coefficient (r)	p-value
Baseline Substrate utilization:			***Body Composition:***		
Resting:			Weight (kg)	-0.385	0.094
RER	-0.212	0.370	BMI ($kg.m^{-2}$)	**-0.477**	**0.033**
Fat oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)	-0.162	0.507	WC (cm)	-0.339	0.144
CHO oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)	**-0.447**	**0.055**	HC (cm)	-0.438	0.053
			BF%	-0.304	0.192
Steady State:			FFSTM (kg)	-0.120	0.615
RER	-0.393	0.087	FM (kg)	-0.335	0.149
Fat oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)	0.243	0.301	Android FM (kg)	-0.263	0.263
CHO oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)	**-0.462**	**0.040**	Gynoid FM (kg)	-0.370	0.109
			SAT (cm^2)	**-0.450**	**0.046**
			VAT (cm^2)	0.068	0.775

Regression Model (ΔVO_{2peak}): R^2=0.375 (Adj R^2=0.297) p=0.023	**Coefficient**	**p-value**	**95% Confidence Interval**
Baseline:			
Steady State CHO oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)	-219.4	0.050	-439.1 to 0.2
Resting CHO oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)	-1,388	0.059	-2,835 to 60

Regression Model (ΔVO_{2peak}): R^2=0.422 (Adj R^2=0.306) p=0.037	**Coefficient**	**p-value**	**95% Confidence Interval**
Baseline:			
Steady State CHO oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)	-206.3	0.065	-427.1 to 14.6
Resting CHO oxidation rate ($mg.min^{-1}.FFSTM^{-1}$)	984.0	0.221	-2,627.3 to 659.3
SAT (area)	-10.6	0.288	-31.2 to 9.9

Note: RER: respiratory exchange ratio; CHO: carbohydrate; FFSTM: fat-free soft tissue mass; SAT: subcutaneous adipose tissue.

3.4 Discussion

This study found high inter-individual variability in the CRF response to a 12 week exercise intervention among sedentary, obese black SA women (±8.4%) in excess of non-exercising CTL, which was considered clinically meaningful. Despite marked differences in CRF response between high and low responders (based on a median split in ΔVO_{2peak} among exercise participants) there were no differences in either baseline VO_{2peak} or in exercise dosage (class attendance and exercise intensity) between groups. However, baseline comparisons between the two groups revealed that HRS were ~11kg lighter. Furthermore, during baseline steady state exercise at 50% $VO_{2peak,}$ HRS showed a preference for fat

oxidation, deriving ~62% of energy expenditure from fat oxidation compared to just 41% in LRS. This translated into higher baseline fat oxidation rates and lower carbohydrate oxidation rates, adjusted for body size (per kg FFSTM). Notably, gynoid FM decreased in the HRS group in response to exercise training, despite no change in dietary intake over the 12 weeks, while in LRS gynoid FM remained unchanged. In non-exercising controls weight, FM and proportional gynoid FM all increased. This finding supports the role of fat oxidation in facilitating body composition improvements in response to exercise training. In contrast, despite no improvements in CRF or body composition, during steady state exercise LRS improved their ability to oxidize fat and reduced their reliance on carbohydrate oxidation over the 12 week exercise intervention and increased S_I compared to non-exercising controls. Around 37.5% of the variability in CRF response to exercise could be explained by baseline variability in carbohydrate oxidation rates both at rest and during steady state exercise with the addition of SAT explaining an additional 5%.

By design, the VO_{2peak} response to 12 weeks of exercise training between HRS and LRS differed widely; HRS increased VO_{2peak} by around ~21% while in LRS this remained unchanged. By comparison non-exercising CTL tended to show a decline in relative CRF ($p=0.075$) and at post-testing VO_{2peak} measures, either adjusted or unadjusted for body size, were below both LRS and HRS. Low CRF response to exercise training, as seen in LRS, is not unusual and is often attributed to inadequate exercise stimulus, with increased volume, intensity or a combination of both, successfully eliminating VO_{2peak} non-response[140,147,150]. However, in this study both LRS and HRS had similar baseline VO_{2peak} and were exposed to similar volume and intensity of exercise. A recent study with a repeat design showed that additional subsequent training load given to non-responders led to improved CRF, which was attributed to haemodynamic changes[150]. However, despite evidence supporting the link between substrate utilization during exercise and VO_{2peak}[151,153,157,265], this and many other studies investigating CRF response variability do not routinely measure substrate utilization during exercise[140,147,148,277]. Given that substrate utilization during exercise indirectly represents skeletal muscle oxidative capacity, it provides a more sensitive measure than resting substrate utilization for assessing muscle adaptations to exercise training. While haemodynamic and other physiological and biochemical changes are not discounted, this study clearly showed group differences in baseline substrate utilization, with baseline carbohydrate oxidation rates (resting and steady state exercise) adjusted for body size explaining 37.5% of the variability in CRF response. HRS demonstrated lower RER, greater

fat oxidation rates and a reduced reliance on carbohydrate oxidation during exercise. As such, greater baseline fat utilization in HRS may have facilitated a superior CRF response to the subsequent 12 weeks of exercise training. Furthermore, the improvements in fat utilization as shown in LRS (reduced RER at post-testing) may then have placed them in a better position to show improved CRF in a subsequent round of exercise training, in line with the findings of the aforementioned study[150]. While we were not in a position to test this, this warrants further investigation.

Following exercise training, resting RER and carbohydrate oxidation rates showed a tendency to increase in HRS, while in LRS resting RER declined, representing an increase in the rate of fat oxidation. The response in LRS is in line with expectations, as higher fat utilization during exercise is likely to reflect an increased fat utilization at rest[162]. However, the tendency for increased carbohydrate utilization at rest in HRS is somewhat unexpected as dietary intake and macronutrient composition remained unchanged throughout the 12 week intervention. Fasting plasma glucose levels were also relatively constant and not associated with resting RER ($p>0.05$). Although not measured in this study, it is possible that the higher RER consistently shown in HRS might be explained by a combination of reduced plasma free fatty acid and increased muscle glycogen content as has been previously shown[163,164,278–280].

Body weight differed between LRS and HRS at baseline, with HRS being ~11kg lighter, putting them in a lower BMI category (Obese class I: 30-34.9kg.m^{-2}) than LRS (Obese class II: 35-39.9kg.m^{-2}). However, in response to the 12-week exercise intervention, while both LRS and HRS reduced WC, compared to CTL (in whom WC increased), reductions in hip circumferences were more pronounced among HRS than LRS. Commensurate with this finding, HRS mobilized FM specifically from gynoid depots rather than VAT depots. This is a novel finding given that meta-analyses typically show mobilization of VAT in response to exercise training, even after 12 weeks of exercise and in the absence of dietary restriction[168]. However, as shown in the previous chapter, baseline substrate oxidation at rest and during exercise explained over 60% of the variability in change in gynoid FM (kg) in this group, again supporting a role for substrate oxidation in body composition changes in response to exercise training. While LRS did improve their ability to utilize fat to fuel during exercise energy expenditure compared to non-exercising controls, this remained lower than that for HRS. This study shows that among obese, sedentary individuals there is high variability in baseline substrate utilization to fuel energy expenditure. Greater capacity to utilize fat may

result in more beneficial body composition changes in response to exercise training, while individuals with lower fat utilization may take longer to see improvements. Further studies are needed to assess this among other age, gender and ethnic groups.

Reductions in gynoid depots specifically in response to exercise training is of interest given the ethnic differences in body fat distribution and association with insulin sensitivity previously reported. Compared to white women, black women have less VAT for a given BMI and waist circumference and carry more FM peripherally in gluteal depots[86]. Furthermore, in black premenopausal women, gluteal and abdominal SAT, rather than VAT, were more closely associated with reduced insulin sensitivity[86], possibly related to their reduced adipogenic gene expression[88], larger cell size and a higher inflammatory profile[89]. While peripheral depots potentially provide a buffer for excess free fatty acids, these findings suggest that peripheral depots may be under increasing stress with cumulative weight gain. In comparison to exercising participants, non-exercising controls increased weight and BMI and FM compared to HRS. This finding is in line with findings from previous longitudinal studies in black SA women[219] and highlights the importance of structured physical activity in preventing weight gain and facilitating weight loss over time in this population group. Exercise training may also be an effective tool for mobilizing fat from these depots, which may ultimately be beneficial in improving insulin sensitivity over the longer term. In this study however, improvements in insulin sensitivity were only significant among LRS and were not associated with changes in CRF, metabolic rate and substrate utilization, at rest or during exercise, in response to exercise training. This is somewhat surprising given the improvements in fat oxidation and body composition shown in HRS. However, it is likely that improvements in oxidative capacity in LRS had a greater impact on insulin sensitivity among LRS given that they started from a far lower baseline.

This is the first study to investigate substrate utilization in sedentary obese black SA women in response to an exercise intervention and it contributes to the growing body of research related to the variability in CRF response to exercise training. Adherence to the prescribed exercise training intervention was good (79.4% of classes attended) and the testing protocol well controlled and standardized. There are however limitations to this study. Outcome measures were only undertaken pre- and post-intervention, so it is not possible to plot the time course of changes observed. The possibility of identifying threshold levels of substrate utilization and oxidation rates that may be required for improvements in CRF or body

composition can therefore not be determined. Under-reporting of dietary energy intake which is shown in Supplementary Table 3.3 was evident. Dietary intake data shows high day-to-day variability and being self-reported is inherently unreliable, however the data was collected by a registered dietitian and every effort was made to check records for completeness. The sample size was also relatively small which may have underpowered the ability to show stronger associations for some variables.

CRF response to 12 weeks of exercise training varied markedly among this group of sedentary, obese, untrained black SA women, despite having similar initial VO_{2peak} and being exposed to similar exercise training stimulus. Notwithstanding a low CRF response, low VO_{2peak} responders were able to improve rates of fat oxidation and reduce reliance on carbohydrate oxidation to fuel exercise, as well as to improve insulin sensitivity. By comparison these variables remained unchanged in non-exercising controls, who also increased body weight and composition and showed reduced daily step count. In contrast, preference for fat oxidation at baseline appeared to have improved the ability of high responders to not only increase CRF, but also to mobilize fat and improve body composition in response to exercise training. Baseline variability in carbohydrate oxidation rates during moderate intensity steady state exercise and at rest explained 37.5% of the variability in CRF response to exercise training in this group. This indicates that variability of substrate utilization to fuel exercise at the outset contributes to the variability in CRF response to exercise training. Screening for substrate utilization during exercise, prior to embarking on an exercise intervention, may assist in identifying individuals that could potentially have a poorer CRF and body composition response to exercise training.

CHAPTER 4

4. Successful and unsuccessful weight-loss maintainers: Strategies to counteract metabolic compensation following weight loss

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For this study I was responsible for advertising and recruitment of participants; arranging laboratory visits; collection and analysis of dietary data; all test procedures for visit 2 including measurements of resting and postprandial metabolic rate and substrate utilization; statistical analysis and interpretation and manuscript write-up.

4.1 Introduction

Global obesity rates are estimated to reach 10.8% in men and 14.9% in women, carrying increased risk for health outcomes[281–283]. Small degrees of weight loss reduce obesity-associated chronic disease risk[284], however weight loss is difficult to achieve and maintain [188,191,285]. None-the-less successful weight-loss maintenance is possible[286,287] and evidence suggests that if weight loss can be maintained for 2-5 years, the chances of relapse are greatly reduced[180,288,289].

Adaptive thermogenesis describes the reduction in total daily EE (TDEE: resting (RMR) and non-resting EE (NREE)) in response to energy deficit, beyond that predicted by changes in body composition, and is implicated in a reduced capacity for ongoing weight loss as well as weight-loss relapse[290–293]. While slower rates of weight loss allow for greater loss of FM and preservation of fat-free mass (FFM), this does not reduce the adaptive decline in RMR following 10% weight loss[294]. With an initial 10% weight loss, RMR and NREE show similar declines, while with a further 10% subsequent weight loss the adaptive response is lower, predominantly affecting NREE[201]. Lower RMR predicts future weight gain independent of BMI or baseline body composition[292,294–296]. Metabolically active FFM (organs and skeletal muscle) does contribute significantly to RMR[297]. Greater body fat percentage (BF%) loss and sparing of FFM during weight loss, lowers body weight regain over the following 2 years[298]. However, in weight-loss maintenance at one year follow-up, decreases in TDEE, NREE and to a lesser extent RMR are shown and correlate with weight loss[208,299]. Given that decreases in all components of EE can be expected in individuals with a history of weight loss, it would be of importance to establish whether this would distinguish them from phenotypically similar individuals with no weight-loss history, increasing their risk for future weight gain.

Metabolic flexibility is the ability to transition between fat and carbohydrate oxidation depending on availability[73]. Metabolic inflexibility increases with obesity and insulin resistance, reducing both fasting fat oxidation and carbohydrate oxidation under insulin stimulated conditions[73]. Formerly obese individuals have shown lower fasting and post-prandial fat oxidation responses compared to matched controls[300,301]. Low fasting fat oxidation increases relative carbohydrate utilization, thus favouring fat storage over oxidation and increasing the risk for greater fat deposition, particularly at times of positive energy balance on dietary relapse[66,188,227,302–306]. Suppressed fat oxidation accompanying prior weight

loss would therefore be likely to increase the risk for future weight gain and fat deposition relative to individuals who had never engaged in periods of weight loss.

Weight-loss associated declines in components of metabolism combined with suppressed fat oxidation at rest and postprandially, predisposes weight-reduced individuals to increased fat storage and subsequent weight regain. We hypothesized that, in light of metabolic changes accompanying weight loss, weight reduced and weight-loss relapsed individuals would exhibit reduced energy expenditure and fat oxidative capacity compared to phenotypic controls with no weight loss history, potentially predisposing them to subsequent weight gain. The primary aim of this study is to compare fasting and postprandial metabolic rate and substrate utilization, both absolute and per kilogram FFM, along with subjective appetite ratings between 1) successfully maintained, weight-reduced individuals; 2) weight-loss relapsed individuals; and their respective BMI-matched controls with no weight-loss history, to establish whether weight-loss history increases their risk for future weight gain. The secondary aim is to compare behavioural factors (dietary intake, eating behaviour and physical activity) and body composition (secondary outcomes) between these groups and to explore their association with RMR, to assess whether these contribute to, or counteract metabolic adaptions to weight-loss.

4.2 Methods

Subject selection and screening

Advertisements were placed at local institutions and on the Sport Science Institute of South Africa (SSISA) website, stipulating that previous weight loss had to be intentional/deliberate, without the use of unregulated products, through lifestyle related approaches (diet and/or exercise), unrelated to stress and/or anxiety and free of eating pathology. Participants were screened and placed into 4 groups. Successful weight reduction is defined as weight loss of ≥10%, maintained for over 12 months with weight fluctuations of 3% considered acceptable[214,307]. Successfully reduced (RED) individuals recruited had lost ≥15% of body weight from a BMI ≥27kg.m^{-2}, maintained weight for over 12 months with 12 month fluctuations of ≤5%. Weight relapsed (REL) individuals (BMI ≥27kg.m^{-2}) had previously lost ≥15% of body weight and regained most/all of this weight. Age and BMI-matched, low-weight stable weight (LSW) controls (BMI ≤27kg.m^{-2}) and overweight/obese stable weight controls (OSW) (BMI ≥27kg.m^{-2}) had no prior weight loss history. Exclusion criteria

included being pregnant or lactating, irregular menstrual cycles (<7 cycles per year/cycle intervals >35days), medical condition and/or condition requiring chronic medication that affected metabolic rate (B2-agonists, beta-blockers, corticosteroids, etc.), weight-loss medication/supplements, diagnosis of thyroid dysfunction or an eating disorder.

The study protocol was approved by the University of Cape Town Faculty of Health Science and Human Research Ethics Committee (HREC 214/2012). Prior to testing, all participants were given full information of test procedures, signed informed consent forms and were at liberty to withdraw at any time.

Study design

Participants attended 2 laboratory visits (see Figure 1 below). During the first visit participants reported in the morning fasted, following an overnight fast (10-12 hours). RMR and substrate utilization, body composition, heart rate and blood pressure were measured. An oral glucose tolerance test (OGTT) was conducted during which time participants completed questionnaires covering socio-economic status, education, psychological status, stress and eating behaviour including the three factor eating questionnaire (TFEQ: Factor I representing dietary restraint; Factor II representing disinhibition of control and Factor III representing hunger)[308]. They were then fitted with accelerometers (ACTi Graph, Shalimar, Florida, USA) to record physical activity over a seven day period.

At the second visit, participants reported to the laboratory in the morning, fasted following an overnight fast (10-12 hours). Fasting RMR (MR1-Figure 4.1 below), RER, and subjective appetite ratings were measured. Participants then ingested a standardized high-fat test meal over ten minutes. Immediately thereafter, RMR, RER, and appetite ratings were measured (MR2) and repeated every hour for three hours (MR3-5). After four hours, appetite ratings were measured and participants consumed a self-selected portion of a standardized lunch and completed the final appetite rating. Amount consumed was covertly measured by weighing before and after portion selection. Participants remained resting, but awake for the duration of the trial, were permitted to watch videos and could drink 250ml of water. With a registered dietitian, participants completed one automated, online, self-administered 24-hour dietary recall (ASA24™, Applied Research Programme, National Cancer Institute, Maryland, USA)[309] and recorded food intake on two additional days.

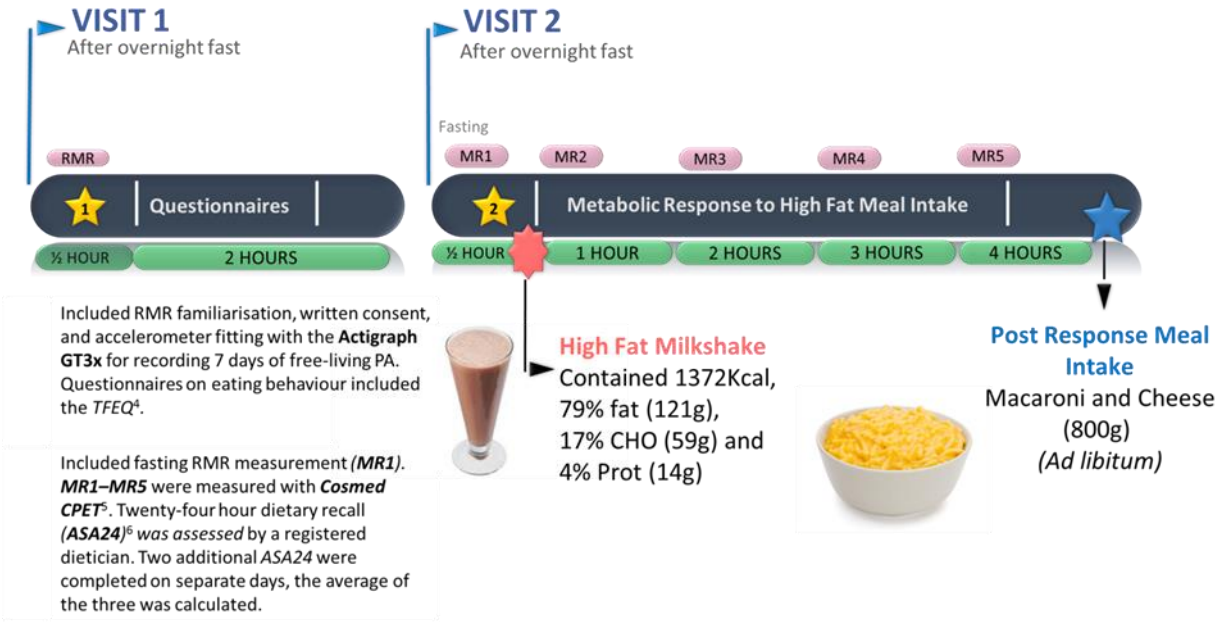

Figure 4.1: Experimental Trial.

NOTE: RMR: resting metabolic rate; PA: physical activity; TFEQ: Three Factor Eating Questionnaire; ASA24: automated online self-administered 24-hour dietary recall; CHO: carbohydrate; Prot: protein.

Anthropometry

Fasted and prior to testing, weight (BW-150, NAGATA, Tainan, Taiwan), height (3PHTROD-WM, Detecto, Missouri, USA), waist and hip circumference were measured. Body composition was measured using Bioelectrical Impedance Analysis (BIA) (Quantum II, RJL Systems, Michigan, USA). The sex-specific prediction equation used was determined by Sun et al (2003) and is recommended for use in epidemiological studies[310]. Participants refrained from drinking water 4 hours prior to the BIA measurement and were requested to void their bladder.

Metabolic rate measurements and calculations:

For both laboratory visits fasting RMR and RER were measured in the morning following a 10-12 hour overnight fast. Reported fasting measures used are those recorded on the second test visit. Participants rested in supine position, in a temperature controlled (21-24°C) room. RMR and RER were measured for 20 min, using the ventilated hood technique (Cosmed Quark CPET, Rome, Italy)[248]. During the second visit this was repeated immediately after consumption of the test meal and repeated every hour thereafter for three hours (i.e. 20min measurement followed by a 40 min rest). Prior to testing the metabolic cart was calibrated

with a Hans Rudolph 3L syringe and analysers calibrated using normal room air (21 % O_2, 4% CO_2, balance nitrogen) and standard gas mixtures (5% CO_2, 16% O_2, balance nitrogen) (BOC Special Gas, Afrox Cape Town, South Africa). RMR, total fat and carbohydrate oxidation were calculated using the equations of Weir [249] and Frayn[250], respectively. Thermic effect of feeding (TEF) was calculated as area under the curve (AUC) for EE after the test meal, expressed as the percentage increase in RMR in response to the test meal (i.e., increase in RMR resulting from processes of digestion, absorption, metabolism, storage and/or elimination of ingested nutrients and/or substrates)[311]. This increase in RMR has also been expressed in absolute terms (Post-prandial EE) over the test period as well as relative to FFM. Post-prandial energy balance (EB) was calculated as the difference between energy ingested in the test meal and post-prandial EE. Post-prandial fat balance was calculated as the difference between fat ingested in the test meal and fat oxidized in the post-prandial period.

75g Oral Glucose Tolerance Test-Blood sampling and analysis:

At visit one following fasting RMR measurements, a cannula attached to a 3-way stopcock was inserted into the antecubital vein. Fasting blood samples (~5 ml) were drawn to determine fasting plasma glucose and insulin. A 75g glucose solution was consumed and blood samples collected at 2 hours. Samples were kept on ice until centrifuged at 3000rpm at 4º C for 10 minutes and subsequently stored at -80º C for later analysis. Plasma glucose concentrations were determined using the glucose oxidase method (YSI 2300 STAT PLUS; YSI Life Sciences, Yellow Springs, Ohio, USA) and serum insulin by direct chemiluminescence immunoassay using the Centaur CPImmunoassay System (Siemenss Medical Solutions Diagnostics, Tarrytown, NJ). Insulin sensitivity was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR)[312].

Test Meal

At visit two following fasting measurements, a liquid test-meal was consumed (milkshake: 5,743kJ (1,372kcal), 200ml cream, 200g full-fat ice-cream, 100ml full-cream milk; 79% fat (120g), 17% carbohydrate (59g), 4% protein (14g)), reflecting an energy dense food available to free-living individuals. Liquid test meals have also been shown to have high reproducibility for postprandial EE[313]. The high-fat content of the test-meal was chosen to optimize detection of metabolic and appetite differences between groups in response to dietary fat. Obese individuals have been shown to be prone to passive overconsumption following energy dense, high fat meals while lean individuals are resistant to this[314]. All

participants consumed a fixed volume of the standardized test-meal, replicating the approach of earlier studies [315,316] and to examine differences in physiological response to the same energy dense meal.

Post-test-meal:

Four hours after the test-meal, participants consumed a self-selected quantity of a standardized lunch (ready-made macaroni cheese 577kJ (138kcal) per 100g, 35% fat, 48% carbohydrate and 17% protein; Woolworth's, South Africa). The container was weighed before and after portion selection and plate wastage measured to determine amount consumed. Time taken to complete the post-test-meal was recorded.

Subjective appetite measurement:

Subjective ratings of appetite (hunger, fullness, desire to eat, prospective food consumption (PFC) and satiety) were recorded before, immediately after the test-meal, every hour thereafter for 4 hours and again following the post-test-meal. A validated visual analogue scale (VAS) was used[317]. Participants placed a mark along a 100mm line representing their subjective rating for each appetite variable, anchored with the opposing extremes of the sensation (e.g. 'Not at all full' (0mm) to 'Extremely full' (100mm)).

Dietary intake – Automated Online Self-Administered 24 hour recall (ASA24)

Dietary intake data was recorded and analysed using the validated, automated online self-administered 24-hour dietary recall (ASA24™, Applied Research Programme, National Cancer Institute, MD, USA) based on the automated multiple pass method[309]. A registered dietitian guided participants through the first 24 hour recall and completed 2 further days. Two week days and one weekend day were recorded to control for variation in intake. The online ASA24 software performs well against interviewer administrated 24-h recall and in comparisons with actual energy and macronutrient intake[318].

Objectively measured Physical Activity

ACTi Graph GT3X (ACTi Graph, Shalimar, Florida, USA) tri-axial accelerometers, fitted at the first laboratory visit recorded habitual physical activity. The device was worn on the right hip for seven consecutive days and removed only during night-time sleep, bathing, showering and swimming. Accelerometer data was processed using ACTi Life Software Version 5 (ACTi Life 5, Pensacola, Florida, USA) whereby wear time was divided from non-wear time.

Non-wear time was recognised as more than 90 minutes or more of consecutive zero counts. Only valid days, defined as at least 10h of wear time, were used in the analyses. A minimum of 4 days and 600 minutes per day was required for data analysis as this is shown to provide 80% reliability[319]. Accelerometer data was then downloaded and exported to Excel data tables using ACTi Life Software Version 5 and analysed for light, moderate and vigorous activity occurring in one minute count intervals[320]. Light, moderate and vigorous intensity physical activity levels were estimated based on accelerometer counts using cut-points according to Matthews[321]. The Matthews equation stipulates counts between 101 and 759 (equivalent to 2-2.9 METs) represent light intensity PA, counts between 760 and 5998 (equivalent to 3-6 METs) represent moderate intensity PA, and counts above 5999 indicate vigorous activity[321]. Energy expenditure was calculated using the PAL method[322].

Statistical Analysis

Sample size determination was based on previous studies in which mean differences (and SD) between lean controls and weight reduced individuals in RER of 0.05(±0.04) and RMR 213(±197)kcal were observed[128,301]. Using a power of 80% and an alfa level of 0.05 an estimated sample size was 8-18 women per group. Data was assessed for normality using histogram plots and the Shapiro Wilk's test, where $p<0.05$ indicated that data was not normally distributed. Mean and standard deviations were reported for normally distributed data and median and inter-quartile-ranges for non-parametric data. This analysis compares RED and REL against their respective phenotypic controls to identify whether, following either weight loss or subsequent weight regain, these individuals return to being indistinguishable from phenotypically similar counterparts with no weight-loss history. Therefore, two group comparisons of normally distributed data was carried out, using two sample t-tests for independent groups with equal variance or a Satterthwaite's independent sample t-tests for unequal variance. Non-parametric data used Wilcoxon Rank Sum tests. Mixed models with repeated measures were used to compare differences in the changes in subjective ratings of appetite between groups. In line with the primary aim of the present study (i.e. to compare BMI-matched individuals), differences between RED versus LSW controls, and REL versus OSW control participants were explored. Pearson's correlation coefficient was used to test for associations between RMR and FFM, both being normally distributed. For all tests significance was accepted at $p<0.05$.

4.3 Results

Participant characteristics, body composition and weight history

There were no differences in body fat percentage (BF%), fat mass (FM) and waist circumference, however RED had greater fat free mass (FFM, p=0.018) and lower waist-to-hip ratio (WHR, p=0.052) compared to LSW (Table 4.1). RED had lost 16.1% (14.4-22.0%) of body weight (BW), maintained for 30 months (12-60 months). REL had lost 19.1% (17.3 – 29.5%) of BW and subsequently regained 21.0% (15.4-26.7%) of BW.

Table 4.1: Participant characteristics and weight loss and regain history

	LSW (n=19)		RED (n=15)		OSW (n=11)		REL (n=11)	
Participant Characteristics								
Age (yrs)	28	25-37	32	26-40	32	29 – 40	34	22 – 41
BW (kg)	59.9	56.8-68.3	67.1	61.5-74.0	87.6	83.4 – 96.0	92.5	79.1-103.3
BMI (kg.m^{-2})	22.7	2.3	24.1	2.3	35.0	4.1	34.1	6.2
BF (%)	29.1	5.2	29.6	4.3	45.4	4.1	43.4	6.6
FM (kg)	18.4	5.2	20.4	5.3	41.6	10.0	42.0	13.5
FFM (kg)	44.0	3.8	47.6*	4.8	49.1	5.3	52.8	5.7
Waist (cm)	68.4	4.8	69.4	5.4	91.8	9.0	90.7	9.6
WHR	0.69	0.68-0.71	0.66	0.64-0.69	0.78	0.74 – 0.83	0.75	0.73-0.81
Weight History								
Weight loss (%BW lost)	-2.8	0.0 - -4.5	-16.1	14.4-22.0	-1.6	0.0 - -3.4	-19.1	17.3- -29.5
Weight regain (%BW regain)	0.0	0.0 – 1.5	0.0	0.0 - 2.8	0.0	1.1 – 2.7	21.0	15.4–26.7
Months at Current Weight (mths)	24	12 – 6	30	12 – 60	9.0	6.0 – 12	6.0	3-30

Note: BW: body weight; BMI: body mass index; BF: body fat; FM: fat mass; FFM: fat-free mass; WHR: waist-to-hip ratio. * indicates p<0.05 for two group comparisons of RED or REL to respective BMI matched controls. Results show mean & SD or median & IQR. Weight loss (**%**BW Lost) = (heaviest adult weight–lightest subsequent weight)/(heaviest adult weight) X 100. %. In the case of RED & REL this would be heaviest weight prior to intended weight loss. Weight regain (%BW Regain) = (Lightest subsequent weight – current weight)/(lightest subsequent weight) X 100.

Metabolic measurements: Fasting & post prandial response

Although fasting and 2 hour plasma glucose levels were similar, the RED had lower fasting insulin concentrations and were less insulin resistant than LSW (Table 4.2). LSW had lower fasting RMR compared to RED, but adjusted for FFM there were no differences between LSW and RED or between OSW and REL. The standard test meal represented a higher energy (131.4 ±10.9 vs. 121.8 ±12.6 kJ/kg FFM, p=0.021) and fat (2.8 ±0.2 vs. 2.6 ±0.3 g/kg FFM, p=0.021) intake per kilogram FFM and energy intake as a percentage of measured

TDEE (94.8% (94.4-95.1%) vs. 94.4% (93.9-94.7%), p=0.015) in LSW compared to the RED, but was not different in comparisons of OSW versus REL ((p=0.137) for both energy and fat intake per kg FFM and p=0.248 for energy intake %TDEE). However TEF, postprandial EE (absolute and per kg FFM), post-prandial energy balance, RER, fat oxidation rate and post-prandial fat balance were similar between LSW and RED and between OSW and REL (p>0.05).

Table 4.2: Metabolic Measurements

	LSW (n=19)		RED (n=15)		OSW (n=11)		REL (n=11)	
Insulin Sensitivity:								
Fasting plasma insulin (ml U.L^{-1})	7.6	4.8-10.5	4.5*****	2.9-5.2	12.4	9.1-16.2	10.8	5.6-14.9
Fasting PG (mmol.L^{-1})	4.8	4.6-5.1	4.9	4.5-5.2	5.4	4.8-6.2	5.2	4.7-7.5
2hr PG (mmol.L^{-1})	6.1	5.3 – 7.3	6.2	5.3–6.7	7.9	6.7–8.9)	7.7	6.1–9.8
HOMA-IR	1.86	1.01-2.43	0.85*****	0.64-1.25	3.10	2.34-4.45	2.36	1.91-3.73
Fasting energy and substrate metabolism:								
RMR (kJ.d^{-1})	5,954	619	6,427*****	732	6,352	1,071	6,619	1,289
RMR.kg^{-1}	135.6	13.0	135.1	10.0	134.3	19.2	130.5	10.9
FFM (kJ.FFM^{-1}.d^{-1})								
RER	0.76	0.04	0.78	0.04	0.78	0.03	0.79	0.04
Post-prandial energy and substrate metabolism:								
TEF (%)	16.4	6.3	13.4	6.9	13.7	9.6	13.4	8.9
Post-prandial EE (kJ)	1,008	100	1,063	146	1,054	197	1,121	176
Post-prandial EE (kJ.FFM^{-1})	23.0	2.1	22.2	2.5	21.8	4.2	21.3	2.5
Post-Prandial EB (kJ)	4,732	100	4,678	146	4,686	197	4,619	176
Post-prandial RER	0.74	0.05	0.75	0.04	0.75	0.04	0.76	0.04
Fat oxidation (mg.min^{-1})	112.1	78.0-129.0	108.6	84.8-124.5	96.4	78.0-154.2	103	86.6-126.5
Post-prandial fat balance (g)	97.0	93.4-104.1	98.4	94.3-102.7	100.3	88.1-104.1	98.9	93.9-103.6
Post-test meal:								
Amount eaten (kJ)	1,481	1,038-2,159	1,435	992-1,887	1,925	1,293-2,222	1,460	1,042-2,397
Amount eaten (%TDEE)	24.6	17.2-35.5	24.1	16.1-31.2	32.7	24.2-36.7	26.3	18.9-36.9
Time taken (min)	10.9	4.8	10.4	3.4	11.4	4.4	7.06*****	2.2

Note: PG: plasma glucose; HOMA: homeostatic model assessment; RMR: resting metabolic rate; RER: respiratory exchange ratio; EE: energy expenditure; EB: energy balance; TEF: thermic effect of feeding.* indicates $p<0.05$ for two group comparisons of RED or REL to respective BMI matched controls. †: LSW vs. RED, $p=0.068$. Numbers with impaired fasting glucose (IFG, fasting glucose ≥ 5.6mmol/l): LSW 2/19, RED 3/15, OSW 5/11 and REL 5/11. Numbers with impaired glucose tolerance (IGT 2h glucose ≥ 7.8mmol/l): RED 1/15, LSW 4/19, OSW 5/11 and REL 6/11. Postprandial EE and RER were calculated as the area under the curve of hourly metabolic measurements following ingestion of the test meal. Post-prandial EB was calculated as the energy intake at the test meal minus post-prandial EE; fat balance was calculated as the fat intake at the test meal minus post-prandial fat oxidation.

Subjective Appetite Response

Subjective appetite measures are shown in Figure 4.2 below, with the LSW and RED compared in graphs in column A and the OSW and REL compared in column B. There were no group differences between LSW and RED (Figure 4.2, column A), however in the

overweight/obese groups (Figure 4.2 column B) the change in 'Desire to Eat' from the fasted state to 60min post ingestion showed a greater decline in the RED (group-by-time, $p=0.033$). For the overweight/obese groups, 'Desire to Eat' (Figure 4.2, B3) and 'PFC' (Figure 4.2, B4) REL show higher subjective ratings in the fasted state (group differences $p=0.003$ & $p=0.023$ respectively), which subsequently decline and remain below fasted levels for the remainder of the trial. OSW however show lower ratings in the fasted state, but the suppression of these ratings, post ingestion of the test meal, is less pronounced than for REL (group by time, $p<0.05$) and at 180min these ratings return to baseline or above fasting levels.

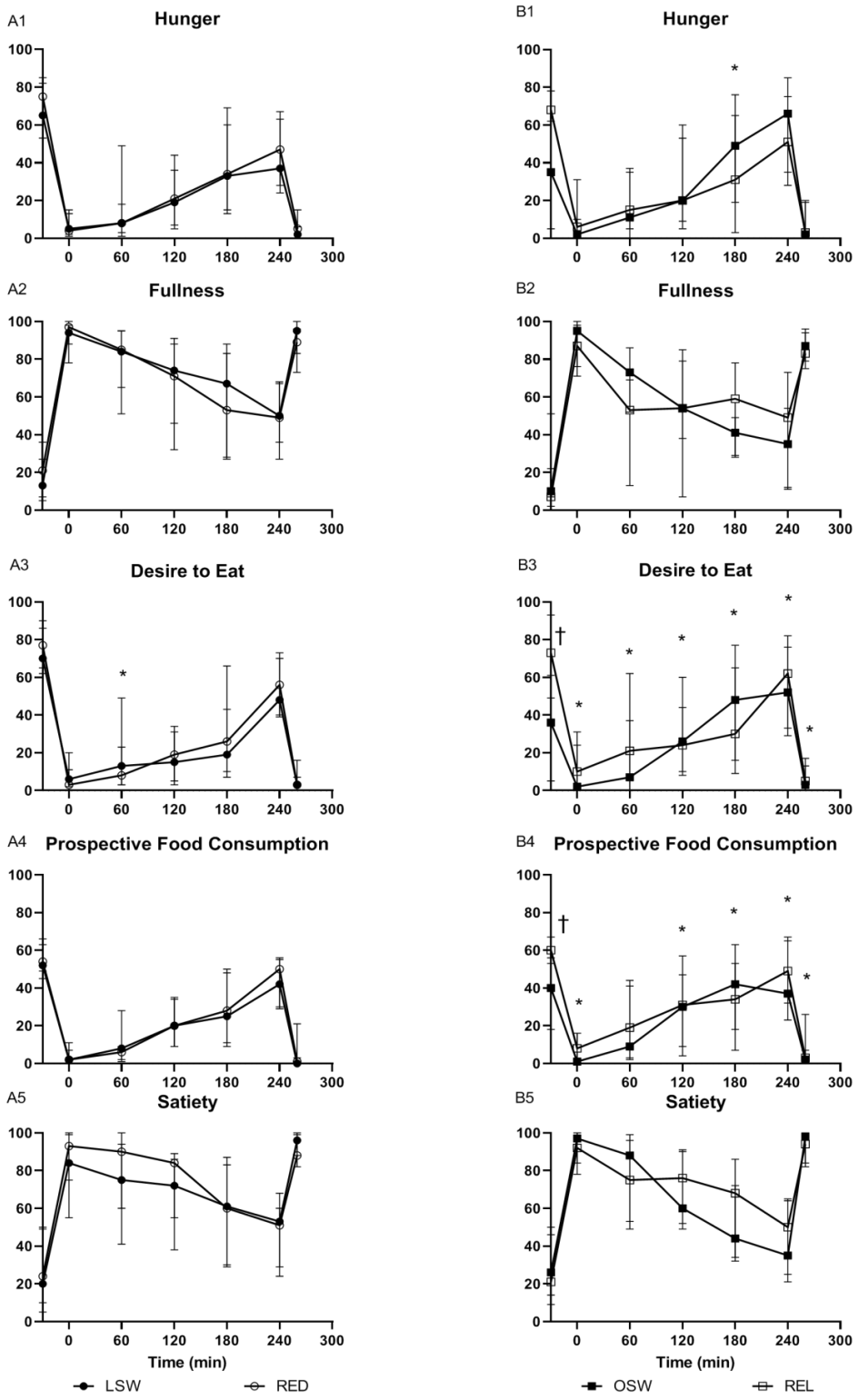

Figure 4.2: Subjective Appetite Measurements for LSW vs RED (column A) & OSW vs REL (column B). NOTE: The time points are as follows: t= -30min (fasting measurement); t=0 was the measurement immediately after consumption of the test meal; t=60, t=120, t=180 & t=240 were the measures recorded every hour after consumption of the test meal; Post TM was the subjective ratings recorded after ad libitum consumption of the post-test meal. PFC: prospective food consumption. Values shown are median and inter-quartile ranges (mm). Group by Time effect: * = $p<0.05$. Group difference: ‡ = $p<0.05$. LSW: lean stable weight; RED: reduced; OSW: overweight/obese stable weight; REL: relapsed.

Post-test meal

LSW and RED consumed similar amounts of the post-test-meal, taking comparable times to complete the meal. While OSW and REL groups consumed similar quantities of food, REL consumed the post-test-meal faster than OSW ($p=0.015$).

Dietary intake, eating behaviour and physical activity

Total daily energy intake (TDEI) for RED and REL did not differ from respective controls (Table 4.3 below). REL tended to report lower TDEI compared to OSW ($p=0.076$) and this became significant when adjusted for FFM ($p=0.017$). RED consumed less carbohydrate and more protein compared to LSW ($p<0.05$), had a tendency for higher fat intake ($p=0.053$). They also reported higher scores for dietary restraint ($p=0.002$) with a tendency for periods of disinhibited eating ($p=0.076$). REL and OSW had similar macronutrient composition and eating behaviour scores.

Although statistically significant, TDEE in RED was only marginally higher than in LSW, however they were less sedentary, did more light ($p=0.024$) and moderate activity (0.032) and a tendency for greater vigorous activity (0.061). REL and OSE had similar levels of TDEE. None of the groups were in energy deficit (TDEI to TDEE ratios), although this ratio higher in OSW compared to REL ($p=0.005$).

Table 4.3: Energy intake and energy expenditure

	LSW (n=19)		RED (n=15)		OSW (n=11)		REL (n=11)	
Dietary Intake & Eating Habits:								
TDEI (kJ.d^{-1})	6,945	5,427-7,912	6,473	5,791-8,619	9104	8,180-11,673	6,577	6,284-9,615
TDEI per kg FFM (kJ.FFM^{-1})	149.4	125.5-170.3	149.0	121.8-179.1	188.7	167.8-236.4	136.0*	114.6-174.9
Fat (% TDEI)	32.1	6.9	36.9	6.8	40.3	7.0	35.4	8.8
Protein (% TDEI)	15.6	4.2	19.2*	6.0	17.4	4.0	19.7	4.2
Protein (g.kg^{-1})	0.96	0.26	1.15*	0.23	1.11	0.33	1.01	0.34
CHO (% TDEI)	53.4	10.0	44.8*	10.3	42.8	8.4	44.8	9.4
TFEQ-Factor I (Restraint)	7.7	4.1	12.1*	3.6	7.0	3.8	9.6	4.8
TFEQ-Factor II (Disinhibition)	5.8	3.9	8.3	3.9	9.5	3.7	11.1	4.2
TFEQ-Factor III (Hunger)	4.8	3.2	5.7	3.7	8.5	3.5	7.4	3.6
Physical Activity:								
Sedentary/Light (kJ.d^{-1})	5,874	5,791-5,975	5,786*	5,586-5,866	5,895	5,849-5,920	5,812	5,619-5,933
Moderate (kJ.d^{-1})	142	59-251	251*	146-402	159	126-205	255	109-402
Vigorous (kJ.d^{-1})	0	0-21	42	0-105	0	0-4	0	0-0
TDEE$^{\#}$ (kJ.d^{-1})	6,054	6,033-6,084	6,079*	6,058-6,113	6,050	6,046-6,058	6,067	6,046-6,088
Energy Balance:								
TDEI:TDEE$^{\#}$	1.08	0.23	1.18		1.53		1.10*	0.10

Note: TDEI: total daily energy intake; FFM: fat free mass; CHO: carbohydrate; TFEQ: Three Factor Eating Questionnaire; TDEE$^{\#}$: total daily energy expenditure determined from ACTi graph measurements. * indicates $p<0.05$ for two group comparisons of RED versus LSW or REL versus OSW. Results show mean & SD or median & IQR.

Associations with RMR

FFM was positively correlated with RMR for the overall group ($r^2 = 0.572$, $p<0.001$) as well as for the RED ($r^2 = 0.765$, $p<0.001$) and REL ($r^2 = 0.634$, $p=0.036$) and approached significance for the LSW ($r^2 = 0.425$, $p=0.070$), but showed no association in OSW. Both TDEE and TDEI were positively associated with RMR ($r^2 = 0.304$, $p=0.030$ & $r^2 = 0.289$, $p=0.031$ respectively). None of the macronutrient intakes (%TDEI) were associated with RMR, however the association between RMR and protein intake in grams per day in RED approached significance ($r^2 = 0.498$, $p=0.058$).

4.4 Discussion

The main findings of this study were that, weight-reduced women had similar RMR (adjusted for FFM) and substrate utilization compared to controls with no weight-loss history. Nevertheless, successful weight-loss maintainers showed behavioural differences that

together, might have counteracted metabolic adaptations to weight loss. These included subjectively indicating greater discipline around dietary intake, reporting increased protein intake and reduced carbohydrate intake, being more physically active and having greater FFM. However, maintaining reduced weight remains challenging over the longer term. Disciplined behaviour, as well as the employment of strategies that bolster both resting and non-resting energy expenditure, is undoubtedly important in reducing metabolic risk for weight regain and supporting successful weight-loss maintenance. Weight-loss relapsed individuals showed no differences in metabolic measurements, but subjectively reported increased appetite in the fasted state. However, following ingestion of a high fat test meal these individuals reported greater reductions in these measures compared to obese/overweight individuals who had never attempted weight loss, suggesting greater drive to consume food in the fasted state but also increased sensitivity to moderating this drive postprandially.

Previously studies have examined energy balance components in order to identify the energy gap that might both explain causes of obesity and help to identify potential remedial action[2,323,324]. Total daily energy expenditure is comprised of RMR (~50-70%), TEF (~5-15%) and NREE (planned and spontaneous physical activity)[110]. Moderate calorie restriction, exercise and maintenance of FFM helps to attenuate the decline in RMR accompanying weight loss, however extreme calorie restriction, even when combined with exercise and maintenance of FFM, results in significant declines in RMR that persist for prolonged periods [110,206,208,325]. Other studies have found that this adaptive response associated with energy restriction, reflects a "transient hypothyroid hypo-metabolic state" that normalizes once energy balance is re-established[209]. In this study weight-reduced individuals successfully maintained goal weight for a median of 30 months. Furthermore, none of the groups studied were in energy deficit and were weight stable at the time of testing. Weight reduced individuals in this study reported consuming more dietary protein compared to controls and this was found to be positively associated with both FFM and RMR, specifically in RED, confirming findings of previous studies[326,327]. RED also report consuming less carbohydrate compared to LSW and similar higher fat, lower glycaemic load weight-maintenance diets are shown to result in smaller declines in EE[211]. Taken together with the fact that RED had greater FFM and were generally more physically active, these factors may explain the lack of relative metabolic disadvantage as a result of prior weight loss. This highlights the importance of incorporating similar strategies within weight maintenance programs to reduce the impact of adaptive thermogenesis and assist in successfully maintaining reduced-weight.

TEF, measured as the percentage increase in RMR following ingestion of a meal, was similar between RED and REL compared to their respective controls. While some studies have reported reductions in TEF following weight loss and stabilization over shorter time periods of 10 days[302], these results show no indication of this in RED or REL groups. It has also been suggested that the TEF is associated with appetite or satiety, although a recent meta-analysis found no evidence to support this[311]. Appetite ratings both fasted and post prandial, are shown to increase immediately after weight loss, remaining at almost identical levels one year later in the weight maintenance phase, despite steady and sustained partial weight regain[328]. Our results however showed no difference in subjective appetite ratings, in the fasted state and postprandially, between RED and LSW, showing that the RED are not at increased risk for weight regain as a result of greater appetite sensations. REL however reported greater appetite drive in the fasted state compared to controls potentially increasing their risk for greater subsequent food intake. However, postprandially they report greater reductions in appetite drive while in controls this rose to levels at or above those reported in the fasted state, which may increase the risk for regular snacking in always overweight/obese, despite prior intake of high fat, energy dense foods.

Objectively measured physical activity showed that RED engaged in more moderate activity, had a tendency for increased vigorous activity and spent less time in sedentary behaviour. NREE, both planned and spontaneous physical activity, declines during weight loss as well as weight-loss maintenance over the following 6 and 12 months, and is associated with subsequent weight regain[213]. However, other studies have demonstrated that while NREE is reduced during weight loss, it recovers during weight maintenance and high levels of physical activity are characteristic of successful weight-loss maintainers, which is in line with these results[208,329]. Consistently maintaining higher levels of moderate and vigorous activity and reducing sedentary behaviour may be key strategies that have assisted the RED group to successfully maintain previous weight loss.

There was no evidence of metabolic inflexibility in RED or REL compared to respective controls, both fasted and in response to the high fat test-meal. RED were significantly less insulin resistant than LSW, characterised by considerably lower fasting insulin levels (see chapter 5 for further commentary[330]). Previous studies show that insulin resistance reduces fasting fat oxidation capacity and increases postprandial fat oxidation under insulin

stimulated conditions[331]. With weight loss, improvements in insulin sensitivity result in lower 24 hour and postprandial fat oxidation, despite enhanced clearance of dietary fat[217,304,332,333], suggesting a preference for fat storage over oxidation in the fasted state. Despite being less insulin resistant, these results show that long term weight reduced individuals display similar levels of fasting and postprandial fat oxidative capacity compared to controls with no weight-loss history. Enhanced insulin sensitivity may also promote weight regain through more efficient uptake and utilization of carbohydrates and storage of dietary fat, with rapid nutrient clearance potentially driving appetite and weight regain on dietary lapse[306,334]. Protein in particular has long lasting suppressive effects on appetite hormones, while carbohydrates tend to act more acutely with subsequent rebounds in appetite[335,336]. Compared to LSW, RED reported greater protein and fat intake and reduced carbohydrate intake, suggesting behavioural adaption that may support appetite control and weight-loss maintenance.

This study was able to identify individuals who had lost a significant amount of weight and either successfully maintained this weight loss for a median of 30 months or regained all of the weight previously lost. This enabled investigation into the longer term effects of weight-loss and regain compared to individuals without a history of weight-loss and regain. Objective measurements of physical activity also avoided issues around over or under reporting. However, there were limitations to the study. Firstly the study was cross sectional and therefore, in line with aims, can only point to associations and make metabolic and behavioural comparisons against matched controls. Secondly, it may be argued that the choice of a liquid test meal may have led to lesser effects on both appetite and thermogenesis. However, our study sought to identify evidence of appetite dysfunction in obesity that might remain present in post obese individuals. This has been identified in other studies where a high fat pre-load led to subsequent passive overconsumption relative to always lean individuals, and could potentially contribute to weight loss relapse[314]. This study relied on self-reported weight history rather than documented weight changes from formal interventions. It also made use of BIA for measurement of body composition, which has limitations. Lastly, we had relatively fewer participants in the REL and OSW groups which may have underpowered the effects shown for these comparisons.

In conclusion, the current study found no differences between reduced weight individuals and always lean controls in terms of resting metabolic rate and substrate oxidation, implying that the reduced weight individuals are not in a metabolic state that will promote weight regain.

This finding contributes to the small body of evidence that not all reduced obese individuals are at a metabolic disadvantage that will promote weight regain[209,302,333]. In weight-loss relapsed individuals metabolic parameters and behavioural strategies were indistinguishable from matched controls, showing that weight-loss associated metabolic disadvantage is likely reversed with weight regain. However weight reduced individuals were less insulin resistant than BMI-matched counterparts, potentially increasing the risk for weight regain with dietary relapse and physical inactivity. They exerted significant conscious behavioural efforts to maintain previous weight loss, reporting greater dietary restraint, manipulating macronutrient components of the diet that supported maintenance of FFM as well as improving satiety and were more physically active compared to controls. Adaptive thermogenesis is shown to accompany weight loss and can be sustained in weight-loss maintenance, reducing all components of TDEE. However, similar metabolic rates shown for components of TDEE in successful weight-loss maintainers compared to controls is likely the result of conscious behavioural strategies that act together to counteract weight-loss associated declines in energy expenditure. This highlights the importance of these strategies as key components in weight maintenance programs following substantial weight loss.

CHAPTER 5

5. Enhanced insulin sensitivity/reduced insulin resistance in successful, long term weight loss maintainers compared to matched controls with no weight loss history

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For this study I was responsible for development of the research question; advertising and recruitment of participants; management of laboratory visits; collection and analysis of dietary data; measurements of resting metabolic rate and substrate utilization; statistical analysis and interpretation and manuscript write-up.

5.1 Introduction

The prevalence of overweight and obesity continues to rise globally, along with associated comorbidities such as T2DM, cardiovascular disease and certain cancers, consequently placing a heavy burden on health care provision[9,284]. This is evident in developed as well as developing countries where obesity is associated with the transition from rural to urban settings[337]. Merely being overweight carries a three-fold increased risk of T2DM[338–340]. Insulin influences energy metabolism and storage through its effect on substrate uptake and utilization along with mobilization of stored energy reserves, operating in a way that preferentially favours carbohydrate metabolism, lipid and glycogen synthesis and storage, and protein synthesis[341]. In certain populations, long-term weight/BMI gain from early adulthood onwards carries an increased risk for development of T2DM even after adjusting for final BMI, suggesting that weight gain itself is associated with impaired metabolic function[340].

Lifestyle factors play a mechanistic role in obesity and disease presentation. Obesity, combined with low levels of physical activity, is associated with intracellular lipid accumulation in skeletal muscle and liver that impairs insulin signalling, reducing skeletal muscle glucose uptake and utilization and weakening insulin-mediated inhibition of hepatic glucose production[123,126,342–344]. Skeletal muscle alterations in response to exercise improve uptake, utilization and storage of glucose, increasing overall capacity for oxidative metabolism and reducing intramuscular lipid content, thus improving overall skeletal muscle metabolic flexibility[176,177,345]. Both resistance and aerobic exercise have also repeatedly been shown to reduce intrahepatic lipid content independent of weight loss[93,346–349]. Regular physical activity is therefore independently associated with reduced insulin resistance and improved insulin sensitivity both in the liver and skeletal muscle.

Weight loss interventions using calorie restriction and/or increased physical activity have shown improvements in insulin sensitivity, with further gains achievable through a combination of both[176,194,350–354]. While some studies show sustained improvements in insulin sensitivity with successful weight maintenance at 12 and 18 month follow-up, other studies have shown either continued improvement or a reversal with weight regain[106,355,356]. It is however unclear whether weight reduced or weight loss relapsed individuals eventually return to a level of insulin sensitivity that is in line with phenotypically similar individuals

with no history of weight gain and loss or whether over the long term they are metabolically worse off as a result of this weight history. The aim of this study is therefore firstly to compare insulin sensitivity of 1) weight reduced individuals, 2) overweight/obese, weight relapsed individuals and 3) BMI-matched controls with no history of weight loss or regain and secondly to identify any factors that might explain variations in insulin sensitivity within this sample.

5.2 Methods

This study used the same female participants (aged 20 – 45 years) recruited, screened and allocated to the same study groups as previously described in Chapter 4 above. Successfully weight reduced (RED) individuals had previously lost ≥15% of their BW from a BMI ≥27kg.m^{-2} and maintained this for over 12 months with ≤5% fluctuation from goal BW over the previous 12 months. Age matched, stable low-weight (LSW) controls had a BMI ≤27kg.m^{-2}, but with no prior weight loss history. Weight relapsed (REL) individuals had a BMI ≥27kg.m^{-2}, having previously lost ≥15% of their BW, but subsequently regained all of this weight. Age matched, overweight/obese stable weight controls (OSW) had a BMI ≥27kg.m^{-2} but no weight loss history. The study protocol was approved by the University of Cape Town Faculty of Health Science and Human Research Ethics Committee (HREC 214/2012). Prior to testing, all participants were given full information of test procedures, signed informed consent forms and were at liberty to withdraw at any time.

Study design

Participants attended the laboratory in a fasted state (10-12 h overnight fast) between 6am to 9am. Height, weight, waist and hip circumferences were measured and body composition was determined using BIA. RMR and substrate utilization were then measured for 20 minutes using the ventilated hood technique (Cosmed Quark CPET, Rome, Italy). RMR and total rates of fat and carbohydrate oxidation were calculated using the equations of Weir[249] and Frayn[250], respectively. Thereafter a 75g oral glucose tolerance test was performed (OGTT) to determine insulin sensitivity/resistance, as described below. Participants then performed an 8-10 minute, single-stage submaximal fitness test to predict VO_{2peak} and completed a number of questionnaires covering medical history, general health, reproductive history, basic socio-demographic as well as weight history, as previously described. Accelerometers (ActiLife 5, Pensacola, Florida, USA) were fitted and instructions given on how to wear these and were

subsequently collected 7 days later to objectively measure habitual physical activity. This data was then analysed for light, moderate and vigorous activity taking place in one minute count intervals (unbouted) using cut-off points according to Matthews[320,357,358], as previously described. A Registered Dietitian guided participants through an online 24 hour food recall (ASA24™, Applied Research Programme, National Cancer Institute, MD, USA) and requested that 2 further 24 hour food recalls were completed (covering one weekend day and 2 weekdays).

Oral Glucose Tolerance Test-Blood sampling and analysis:

Following fasting RMR measurements, a cannula attached to a 3-way stopcock was inserted into the antecubital vein for blood sampling. A fasting blood sample (~18 ml) was drawn for the determination of fasting plasma glucose and insulin. Thereafter the participants consumed a 75g glucose solution and blood samples were collected at 2 hours. Samples were kept on ice until centrifuged at 3000prm at 4º C for 10 minutes and subsequently stored at -80º C for later analysis. Plasma glucose concentrations were determined using the glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA). Commercial radio immunoassays were used to measure plasma insulin (Axsym Insulin Assay, Abbott Laboratories, Illinois, USA). Insulin resistance (IR)/sensitivity was estimated using the Homeostasis Model Assessment (HOMA-IR, using fasting glucose and insulin measures) and the insulin sensitivity index ($ISI_{(0,120)}$, using fasting and 120 minute glucose and insulin values) reflecting hepatic IR and peripheral insulin sensitivity respectively[312]. HOMA-IR and $ISI_{(0,120)}$ were determined using the following formulae:

- HOMA-IR = (fasting glucose ($mmol.L^{-1}$) x fasting insulin ($mU.L^{-1}$))/22.5;
- $ISI_{(0, 120)}$ = mean clearance rate/log mean serum insulin; where mean clearance rate = (75000mg + (0min glucose-120min glucose) x 0.19 x BW (kg)/120min)/mean plasma glucose) as validated by Gutt et al (2000)[359].

Statistical Analysis

Data was assessed for normality using histogram plots and the Shapiro Wilks test, where $p<0.05$ indicated that data was not normally distributed. For normally distributed data mean and standard deviation were reported and a one-way ANOVA was used for comparisons across all four groups with a Bonferroni post-hoc test to identify significant differences between individual groups. For non-parametric data median and inter-quartile-ranges were

reported, a Kruskal-Wallis (nonparametric) ANOVA was used for multiple comparisons and differences between groups were assessed using a Wilcoxon rank-sum test using a Bonferroni adjustment of p-value for multiple comparisons (significance at 0.008, $\alpha = 0.05/6$ tests). Both HOMA-IR and $ISI_{0,120}$ were log transformed to give a normal distribution. Pearson's correlation coefficient and simple linear regression models were used to test for associations between variables and log HOMA-IR and log $ISI_{0,120}$ respectively. Multiple linear regression was used to model predictors of variability in HOMA-IR and $ISI_{(0,120)}$. The multiple linear regression models were tested for normality of residuals, linearity and homoscedasticity. Outliers were checked for influence and leverage and multicollinearity of predictors was assessed using the variance inflation factor (VIF > 5). For all tests a p-value < 0.05 was considered statistically significant.

5.3 Results

Participant characteristics

Participant characteristics and weight history are presented in Table 5.1. RED and REL were not different to LSW and OSW respectively. By design LSW and RED had lower body weight and BMI and consequently showed reduced FM, FFM, BF% and waist circumference compared to the OSW and REL.

Table 5.1: Participant characteristics and weight history

	LSW (n=19)	RED (n=15)	OSW (n=11)	REL (n=11)
Participant characteristics				
Age (years)	28 (25-37)	32 (26-40)	32 (29-40)	34 (22-41)
Body Weight (kg)	59.9 [D,E] (56.8-68.3)	67.1 [B,C] (61.5-74.0)	87.6 (83.4-96.0)	92.5 (79.1-103.3)
Body Mass Index (kg.m^{-2})	22.7±2.3 [D,E]	24.1±2.3 [B,C]	35.0 ±4.1	34.1 ±6.2
Body fat (%)	29.1±5.2 [D,E]	29.6±4.3 [B,C]	45.4 ±4.1	43.4 ±6.6
Fat mass (kg)	18.4±5.2 [D,E]	20.4±5.3 [B,C]	41.6 ±10.0	42.0 ±13.5
Fat-free mass (kg)	44.0±3.8 [D,E]	47.6±4.8	49.1 ±5.3	52.8 ±5.7
Waist (cm)	68.4±4.8 [D,E]	69.4±5.4 [B,C]	91.8 ±9.0	90.7 ±9.6
Waist-to-hip ratio	0.69 [D,E] (0.68-0.71)	0.66 [B,C] (0.64-0.69)	0.78 0.74-0.83)	0.75 (0.73-0.81)
Weight History				
Weight loss (%BW lost)	-2.8 (0.0 – 4.5)	-16.1 [A B] (14.4 – 22.0)	-1.6 (0.0 – 3.4)	-19.1 [E F] (17.3 - 29.5)
Weight regain (%BW regain)	0.0 (0.0-1.5)	0.0 (0.0-2.8)	0.0 (0.0 – 2.7)	21.0 [C E F] (15.4 – 26.7)
Months at Current Weight (months)	24.0 [D E] 12.0 – 60.0	30.0 [B C] 12.0 – 60.0	9.0 6.0 – 12.0	6.0 3.0 – 30.0

Note: Significant differences (p<0.05): A: LSW & RED; B: RED & OSW; C: RED & REL; D: LSW & OSW; E: LSW & REL; F: OSW & REL

Dietary intake, physical activity and metabolic measurements

Table 5.2 shows that OSW consumed significantly more energy than LSW (p<0.001) and RED (p=0.003), but when adjusted for BW this was not significantly different. The LSW consumed more carbohydrates and less fat than the other three groups, although only significant between the LSW and OSW. There were no significant differences in sedentary, light and moderate activity. The RED engaged in more vigorous activity compared to the other three groups (p=0.050), but this was only significant between the RED and OSW. In terms of overall fitness, the LSW had higher peak oxygen consumption (VO_{2peak}) compared to OSW (p<0.05) and the RED was higher than both OSW and REL (p<0.05) while RED and LSW were not different (p>0.05). There were no differences in RMR or substrate utilization between the groups.

Table 5.2: Dietary intake, physical activity and metabolic measurements

	LSW (n=19)	RED (n=15)	OSW (n=11)	REL (n=11)
Dietary Intake				
Energy (kJ.d^{-1})	6,947 ^{D}	6,474 ^{B}	9,107	6579
	5428-7914	5792-8621	8182-11676	6286-9617
Energy (kJ.kg^{-1})	106.7 ±18.8	106.7±29.7	107.1 ±23.4	87.9 ±35.2
Fat (% Total Energy)	32.1 ±6.9 ^{D}	36.9 ±6.8	40.3 ±7.0	35.4 ±8.8
CHO (% Total Energy)	53.4 ±10.0^{D}	44.8 ±10.3	42.8 ±8.4	44.8 ±9.4
Protein (% Total Energy)	14.0	20.2	17.2	18.8
	12.5-18.2	12.9-23.7	14.4-20.6	15.9-23.3
Protein (g.kg^{-1})	0.96 ±0.26	1.15 ±0.23	1.11 ±0.33	1.01 ±0.34
Physical Activity				
$VO_{2\ peak}$ (ml.min^{-1}.kg^{-1})	37.4 ±6.3	39.9 ±4.9	30.2 ±3.8	33.1 ±5.9
Sedentary Time (min.d^{-1})	1186	1118	1155	1172
	1098-1208	1038-1192	1104-1179	1104-1216
Light Activity (min.d^{-1})	177	209	210	167
	158-247	172-275	173-249	162-205
Moderate Activity (min.d^{-1})	79	111	86	93
	58-107	73-127	79-110	67-131
Vigorous Activity (min.d^{-1})	0	4.1^{B}	0	0
	0-0	0-11.3	0-5	0-0.125
Metabolic Measurements				
RMR (kJ.d^{-1})	5,954 ±619	6,427 ±732	6,352 ±1071	6,616 ±1289
RMR.kg FFM^{-1} (kJ.FFM^{-1}.d^{-1})	135.6 ±13.0	135.2±10.0	134.3±19.2	130.5 ±10.9
RER	0.76 ±0.06	0.76 ±0.06	0.78 ±0.04	0.79 ±0.05

Note: Significant differences ($p<0.05$): A: LSW & RED; B: RED & OSW; C: RED & REL; D: LSW & OSW; E: LSW & REL; F: OSW & REL. FFM – fat free mass. CHO: carbohydrate; VO_{2peak}: peak volume of oxygen consumption; RMR: resting metabolic rate; RER: respiratory exchange ratio; FFM: fat free mass.

Fasting and 2h plasma glucose and insulin, HOMA-IR and $ISI_{(0,120)}$

Results of the 75g OGTT (Table 5.3) shows that while blood glucose levels are largely comparable across all groups, the RED have significantly lower fasting and 2 hour insulin levels compared to all other groups ($p<0.005$). Across all participants, eight individuals recorded fasting PG ≥7.0mmol/l, a diagnostic criteria for T2DM, despite having previously had fasting blood glucose levels <7.0mmol/l at screening. Of these, two subsequently recorded 2hr PG levels ≥11.1mmol/l, which is a diagnostic criterion for T2DM. Removing these individuals from the analysis did not alter the results significantly.

Table 5.3: Fasting and 2 hour plasma glucose and insulin measurements

	LSW (n=19)	RED (n=15)	OSW (n=10)	REL (n=11)
Fasting Plasma Glucose (mmol.L^{-1})	4.8 (4.6-5.1)	4.9 (4.5-5.2)	5.4 (4.8-6.2)	5.2 (4.7-7.5)
2h Plasma Glucose (mmol.L^{-1})	6.1^{D} (5.3 – 7.3)	6.2$^{B\,C}$ (5.3 – 6.7)	7.9 (6.7 – 8.9)	7.7 (6.1 – 9.8)
Fasting plasma insulin(ml U.L^{-1})	7.6 (4.8-10.5)	4.5$^{A\,B\,C}$ (2.9-5.2)	12.4 (9.1-16.2)	9.3 (5.6-14.9)
2h plasma insulin (ml U.L^{-1})	41.1^{D} (25.0-64.3)	19.7$^{A\,B\,C}$ (10.9-31.1)	91.5 (52.1-140.2)	49.9 (31.2-115.7)
Impaired Fasting Glucose: Fasting Plasma Glucose ≥ 5.6 <7.8mmol.L^{-1} (n (% of category))	2 (10.5)	3 (20)	5 (50)	5 (45)
Impaired Glucose Tolerance: 2h Plasma Glucose ≥ 7.8mmol.L^{-1} (n (% of category))	4 (21)	1 (7)	5 (50)	6 (55)

Note: PG: plasma glucose. Significant differences ($p<0.05$): A: LSW & RED; B: RED & OSW; C: RED & REL; D: LSW & OSW; E: LSW & REL; F: OSW & REL.

RED were significantly more insulin sensitive than all other groups (Figure 5.1). This was shown (Figure 5.1A) using fasting values and determining IR as measured by HOMA-IR (RED 0.85 (0.64-1.25), LSW 1.86 (1.01-2.43), REL 2.36 (1.91-3.73), OSW 3.10 (2.34-4.45); $p<0.001$ for all comparisons with RED). REL were not different to either the LSW ($p=0.138$) or OSW ($p=0.324$). LSW had lower HOMA-IR values compared to OSW ($p=0.015$) while both the OSW and REL were not different ($p>0.05$). The same result was shown in Figure 5.1B using both fasting and two hour values as determined by $ISI_{(0,120)}$ (RED 115.1 (89.8-134.7), LSW 80.0 (66.1-96.4), REL 58.7 (56.2-69.7), OSW 55.7 (43.7-59.9); $p<0.001$ for all comparisons with RED). LSW were more insulin sensitive compared to both OSW and REL ($p<0.05$), while the REL and OSW were not different on either measure ($p>0.05$).

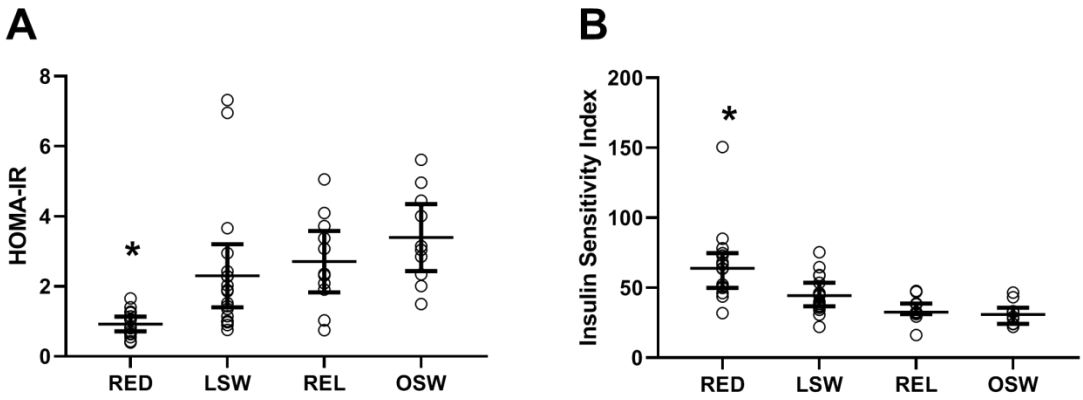


Figure 5.1: HOMA-IR and Insulin Sensitivity Index for RED, LSW, REL and OSW

Note: * indicates RED significantly different compared to all other groups ($p<0.001$).

Associations and regression models for predictors of log HOMA-IR and $ISI_{(0,120)}$

The total sample was analysed to identify significant associations of variables against both log HOMA-IR and $ISI_{(0,120)}$ (see Table 5.4 below).

Table 5.4: Associations with log HOMA-IR and log $ISI_{(0,120)}$

	Correlation coeff.			Correlation coeff.	
	Log HOMA-IR	**Log $ISI_{(0,120)}$**		**Log HOMA-IR**	**Log $ISI_{(0,120)}$**
Weight Loss History:			***Dietary:***		
Body Weight Lost (%)	-0.291†	0.253	**Protein ($g.kg^{-1}$)**	-0.276†	0.158
Body Weight regained (%)	0.245	-0.319†	***Physical Activity:***		
Body Composition:			**Sedentary time ($min.d^{-1}$)**	0.285†	-0.109
BMI (kg/m^2)	0.477*	-0.436*	**Light activity($min.d^{-1}$)**	-0.302†	0.124
WC (cm)	0.481*	-0.479*	**Vigorous activity($min.d^{-1}$)**	-0.349†	0.263
HC (cm)	0.407†	-0.457‡	**VO_{2peak} ($ml.^{-}1.kg.min^{-1}$)**	-0.429‡	0.431‡
WHR	0.362‡	-0.341*	***Substrate Utilization:***		
Fat mass (kg)	0.468*	-0.417†	**Fasting RER**	0.338†	-0.311†
Body Fat (%)	0.523*	-0.508*			
FFM (kg)	0.165	0.044			

Note: † : $p < 0.05$; ‡: $p < 0.005$; *: $p < 0.001$. Log HOMA-IR: Body Weight regained: p= 0.071. Log $ISI_{(0,120)}$: Body Weight lost: p=0.063; Vigorous activity: p=0.065. Coeff: coefficient, BW: body weight; BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio. VO_{2peak}: peak volume of oxygen consumption.

The regression models (Table 5.5 below) were able to predict 61.4% ($p<0.001$) and 59.7% ($p<0.001$) of the variability in log HOMA-IR (Model 1) and log $ISI_{(0,120)}$ (Model 2) respectively in this sample. Using beta coefficients, the strongest predictors in Model 1 were % BW lost (beta -0.508) followed by %BW regained (beta 0.314) and RER ratio (beta 0.298), while for Model 2 %BW lost (beta 0.612), %BW regained (beta -0.600) and RER ratio (-0.231) respectively. Light activity contributed to Model 1 but was not a strong predictor in Model 2, while for Model 2 WHR was a stronger predictor than BF%. Removing the two individuals who exceeded diagnostic criteria for T2DM from the analysis did not significantly alter these results.

Table 5.5: Regression Models to explain variability in log HOMA-IR and log $ISI_{(0,120)}$

	Model 1: Predicting log HOMA-IR				Model 2: Predicting log $ISI_{(0,120)}$			
Predictors:	**Coeff**	**95% CI**		**p-val**	**Coeff**	**95% CI**		**p-val**
		Lower	**Upper**			**Lower**	**Upper**	
% Body Weight lost	-0.038	-0.058	-0.018	<0.001	0.025	0.015	0.036	<0.001
% Body Weight regained	0.029	0.002	0.056	0.037	-0.031	-0.045	-0.016	<0.001
Light activity($min.d^{-1}$)	-0.003	-0.006	-0.001	0.048				
Vigorous activity ($min.d^{-1}$)	-0.033	-0.061	-0.005	0.023	0.015	0.001	0.029	0.043
% Body Fat	0.019	-0.002	0.040	0.073				
RER	4.223	1.326	7.120	0.005	-1.823	-3.372	-0.275	0.022
Waist-to-hip ratio					-1.273	-2.814	0.268	0.103
Constant	-2.406	-4.589	-0.222	0.032	6.499	4.914	8.084	<0.001
Observations:	**50**				**50**			
R^2 (Adjusted R^2)	**0.614 (0.560)**				**0.597 (0.551)**			
p-value	**<0.001**				**<0.001**			

Note: Coeff: coefficient; CI: confidence interval; RER: respiratory exchange ratio.

5.4 Discussion

This study compared metabolic, physiological and lifestyle variables across 4 groups of women classified exclusively according to weight status and weight loss history. The main finding from this study showed that successfully maintained, weight-reduced individuals were significantly more insulin sensitive compared to all other groups investigated. What is remarkable is that women in the RED had maintained substantial weight loss of around 15% for a lengthy period (median 30 months (12-60 months)), and yet were found to be less IR, based on HOMA-IR measures, and more insulin sensitive, based on $ISI_{(0,120)}$ measures, relative to phenotypically similar, age and BMI-matched controls with no weight loss history. There were no significant differences in metabolic rate, substrate utilization or dietary intake that might explain the enhanced insulin sensitivity in this group, besides a modest engagement in vigorous activity. Multiple linear regression models were able to explain 61.4% of the variability in log HOMA-IR ($p<0.001$) and 59.7% in log $ISI_{(0,120)}$ ($p<0.001$) in the total sample. In these models, percentage BW lost and percentage BW regained were significant predictors of insulin sensitivity along with the ability to metabolise more fat in the fasted state. Physical activity, particularly vigorous activity, was also associated with greater insulin sensitivity (light activity was a significant predictor only in Model 1 predicting variability in log HOMA) while none of the dietary intake variables were found to be significant predictors.

Weight loss intervention studies have shown sustained improvements in glucose regulation at 6 month follow-up, but longer term, with weight regain these improvements were reversed[356]. However, other groups have demonstrated that improved insulin sensitivity following weight loss was retained and even enhanced despite weight regain, and here the continued increase in adiponectin and insulin-like growth factor 1, along with no change in visceral fat, were highlighted as possible explanations for this[106]. Our results show that individuals, who have undergone meaningful weight loss in excess of 15% of BW and subsequently maintained this well beyond one year and up to 5 years, are less IR/more insulin sensitive than age and BMI-matched controls. However, compared to RED, the REL group demonstrated increased IR/lower insulin sensitivity, as well as greater 2 hour insulin levels in the 75g OGTT, supporting existing evidence that metabolic benefits that accompany weight loss are not present in subjects who have regained weight. It is interesting to note that individuals who had always been lean, and had not gone through the process of weight loss, were not significantly less IR/more insulin sensitive than the REL, despite having significantly lower BMI. Undoubtedly physiological and metabolic parameters, along with lifestyle choices play an important role in improving metabolic health[351,360]. However, it is also important to recognise that individuals who have a history of substantial weight loss are evidently more insulin sensitive than those who have no weight loss history.

In addition to much higher $ISI_{(0,120),}$ the RED displayed very low HOMA-IR values, with a tight clustering of individuals falling in the bottom half and even below suggested 95% reference cut points[361]. It is therefore pertinent to consider the implications of this, particularly whether very high levels of insulin sensitivity could potentially predispose individuals to subsequent weight regain. In obese, insulin resistant populations higher relative insulin sensitivity is associated with increased prospective weight gain[362,363]. Greater weight regain following weight loss has also been shown in individuals who subsequently consumed a high glycaemic load diet[364]. Changes in adipose tissue histology may also play a role as weight regain after sustained weight loss is accompanied by adipocyte hyperplasia[306]. The greater number of smaller, newly reduced adipocytes are potentially more insulin sensitive, with lower rates of lipolysis and increased rates of fat storage following weight loss[190]. This would suggest that weight cycling increases the number of adipocytes, thereby reducing the inflammatory profile of adipose tissue and improving insulin sensitivity, but also increases the efficiency and capacity for fat storage, thus raising the risk for future weight regain[190]. This emphasizes the importance of dietary recommendations and support in the weight

maintenance phase following substantial weight loss in order to retain metabolic improvements.

Exercise has beneficial effects on body composition and improves insulin sensitivity through enhanced oxidative capacity and reduced intramuscular triglycerides that impair insulin signalling within the cell[176,342,344]. Weight loss incorporating diet and aerobic exercise confers further beneficial effects on insulin sensitivity at one year follow-up that is independently associated with improved cardiovascular fitness[365]. Both light- and vigorous activity were significant predictors of IR as measured by HOMA-IR. There was also a strong negative correlation between sedentary time and light activity and together this demonstrates that increasing light activity at the expense of sedentary time was associated with greater levels of fasting insulin sensitivity as measured by HOMA-IR, which is indicative of lower hepatic IR. It is possible that with sedentary behaviour the reduced number of muscle contractions reduces skeletal muscle glucose clearance as well as lipoprotein lipase (LPL) activity, resulting in reduced triglyceride clearance, thus potentially increasing ectopic fat deposition which would include the liver[366]. Small amounts of vigorous activity predicted improved insulin sensitivity which is supported by interventions showing improvements in insulin sensitivity after just 2 weeks of short duration sprint interval training[367]. Exercise improves fasting fat oxidation which in turn improves insulin sensitivity and is confirmed in our model whereby lower fasting RER significantly predicted greater insulin sensitivity[267]. All groups met the American College of Sports Medicine (ACSM) Guidelines of 150 minutes per week of moderate to vigorous activity and this remains an important component of daily physical activity. However, explicitly incorporating small amounts of vigorous activity may further improve the effect of exercise on insulin sensitivity. All participants undertook large amounts of moderate activity per day, but this did not contribute to insulin sensitivity, possibly indicating that it was lower intensity, intermittent activity rather than more structured higher intensity activity over a continuous period of time.

Dietary intake variables were not found to be predictors of insulin sensitivity. Other studies, considering dietary glycaemic load and macronutrient composition, have also found no association with insulin sensitivity[368–371]. Other components of food intake and diet quality may be more predictive of insulin sensitivity than macronutrient composition per se[372]. Obtaining accurate dietary intake information is inherently problematic, with day-to-day variability making it difficult to determine habitual dietary intake from three 24-hour

recalls[373]. There is also some evidence of inter- and intra-individual variability in glycaemic response to the same food thereby potentially eliciting a variable insulin response[374,375].

One of the strengths of this study was the ability to identify individuals who had undergone substantial weight loss and either successfully maintained this weight loss for periods in excess of a year or regained all of the weight previously lost. This enabled an investigation into the longer term effects of weight loss and regain in comparison to individuals with no weight loss history. Actual measurements of physical activity also avoided issues around over or under reporting. However, limitations of the study included it being cross sectional and therefore only able to point to associations rather than cause and effect. There were also relatively fewer participants in the REL and OSW groups due to difficulty of recruitment of individuals matching the study criteria and this may have underpowered the effects shown. Future studies should analyse the dietary data in more detail, perhaps using indices and their components to assess associations with dietary intake and insulin sensitivity. It would also be of interest to consider mechanisms that might explain the improved insulin sensitivity that accompanies weight loss over the longer term.

In conclusion, successfully weight-reduced individuals, maintaining reduced weight for extended periods of time, are more insulin sensitive than their BMI-matched controls with no weight loss history, independent of dietary intake and physical activity. With weight loss relapse these metabolic benefits are no longer evidenced. Being physically active, engaging in light activity rather than being sedentary and in particular including small amounts of vigorous physical activity predicted greater insulin sensitivity/reduced IR. Weight loss maintenance programs should therefore be emphasized in the period following substantial weight loss in order to retain these benefits. Research is needed to consider dietary strategies that can facilitate weight loss maintenance in light of the enhanced insulin sensitivity, not just in the immediate period following weight loss, but over the long term.

CHAPTER 6

6. Summary and Conclusions

6.1 Summary of Findings & Conclusions

Obesity treatment requires approaches that target the reduction of body weight and fat mass as well as the improvement of CRF, metabolic flexibility and insulin sensitivity. Energy deficit created through exercise represents an approach that could improve many of these variables. Once achieved however, weight loss maintenance becomes increasingly important, but overcoming the metabolic adaptation to weight loss remains a key challenge in obesity treatment. The aims of this thesis were therefore to explore the role of metabolic rate and substrate utilization in influencing changes in body weight, body composition, CRF and insulin sensitivity (see Figure 6.1 below). In Part 1 of the thesis I hypothesised that metabolic rate and substrate utilization in a sedentary obese population will be beneficially altered through exercise training, and that these changes would be associated with improvements in CRF, body composition and insulin sensitivity. In Part 2, I hypothesized that metabolic rate and substrate utilization will be altered through a history of weight loss/regain, predisposing these individuals to weight regain and impairing successful weight loss maintenance. It was also hypothesized that in successful and unsuccessful weight loss maintainers insulin sensitivity would return to levels observed in BMI matched controls without a weight loss history.

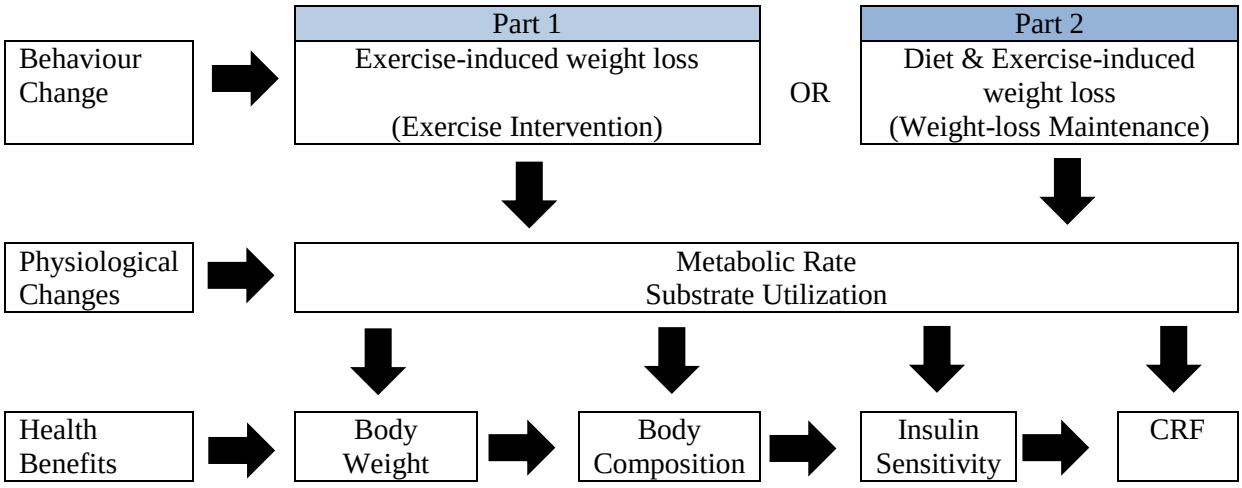


Figure 6.1: Study of the role metabolic rate and substrate utilization in changes in body weight, body composition and insulin sensitivity

PART 1

Summary of findings

Part 1 presented results from the first supervised 12 week exercise intervention in obese black SA women. This study was also the first to measure resting and steady state metabolic rate and substrate utilization at baseline and post-intervention in this population group (See Figure 6.2 below). Black SA women are shown to spend a significant amount of time in sedentary behaviour, while engaging in little or no vigorous activity[220]. They consequently display very low cardio-respiratory fitness (CRF) and are at high risk for obesity, insulin resistance and T2DM[220]. In chapter 2, I showed that 12 weeks of exercise training significantly increased CRF, albeit from a low base (below 20th percentile shown in African American women[282]). Increased CRF enabled an increase in energy expenditure during moderate intensity steady-state exercise (50% of VO_{2peak} at post-intervention) which was fuelled by higher fat oxidation rates, while carbohydrate oxidation rates remained the same. In contrast, the control group showed no change in any of these measures. Resting metabolic rate (RMR) was unchanged in response to exercise training and likely reflected unchanged FFSTM, a major driver of resting EE[39]. Exercise participants reduced body weight, BMI, waist and hip circumference in response to exercise training, whereas in non-exercising controls weight, BMI and waist circumference increased. It is noteworthy that in contrast to findings from other exercise interventions[168], visceral adipose tissue remained unchanged in response to the exercise intervention. Rather, gynoid FM decreased in the exercise group in response to exercise training and increased in controls. Using regression models, we showed that higher baseline fat oxidation rates during exercise and lower baseline resting carbohydrate oxidation rates explained over 60% of the variability in change in gynoid fat mass in response to 12 weeks of exercise training. These results support a role for substrate utilization in achieving body composition improvements in response to exercise training.

EXERCISE INTERVENTION STUDY

Recruited & Randomized

Control (n=15) Age: 23y (21-27) BMI: 33.4 ±2.7 kg.m^{-2}		**Exercise (n=20)** Age: 22y (21-24) BMI: 34.1 ±2.8 kg.m^{-2}
Control	**BASELINE**	**Exercise**
23.9 ± 3.0	VO_{2peak} (ml.min^{-1}.kg^{-1}) (Age associated norm: 35-40 ml.min^{-1}.kg^{-1})	24.9 ± 2.4
Continued habitual activity & dietary patterns		12 Week Exercise Intervention (4d.wk^{-1}) Attendance: 79.4% Exercise intensity: 79.7±4.0% HR_{peak}

Control	**POST TESTING**	**Exercise**
No change	**VO_{2peak} (ml.min^{-1}.kg^{-1})**	↑ (+10.8%)
	Steady State exercise: Same absolute workload	
No change	-% VO_{2peak}	↓
No change	-% HR_{peak}	↓
No change	-Fat oxidation rate (mg.min^{-1} .FFSTM^{-1})	No change
No change	-CHO oxidation rate (mg.min^{-1} .FFSTM^{-1})	No change
	Steady State exercise: Same relative intensity	
No change	-Energy expenditure	↑
No change	-Workload (treadmill gradient)	↑
No change	-Fat oxidation rate (mg.min^{-1} .FFSTM^{-1})	↑
No change	-CHO oxidation rate (mg.min^{-1} .FFSTM^{-1})	No change
	Resting:	
No change	-Energy expenditure	No change
No change	-Fat oxidation rate (mg.min^{-1} .FFSTM^{-1})	No change
No change	-CHO oxidation rate (mg.min^{-1} .FFSTM^{-1})	No change
	Anthropometry	
↑	Body weight (kg)	↓
↑	BMI (kg.m^{-2})	↓
↑	Waist circumference (cm)	↓
No change	Hip circumference (cm)	↓
↑	Waist-to-hip ratio	↓
	DXA-derived Measurements	
No change	Fat Mass (kg & %total fat mass)	No change
No change	Fat Free Soft Tissue Mass (kg & %total fat mass)	No change
No change	Android Fat Mass (kg & %total fat mass)	No change
↑	Gynoid Fat Mass (kg)	↓
No change	Gynoid Fat Mass (%)	↓
No change	Subcutaneous Adipose Tissue (cm^2)	No change
No change	Visceral Adipose Tissue (cm^2)	No change
	Fasting glucose, insulin & Insulin Sensitivity	
No change	Fasting blood glucose	No change
No change	Fasting Insulin	No change
No change	S_I	↑

Figure 6.2: Summary of findings for Chapter 2

Note: BMI: body mass index; VO_{2peak}: peak volume of oxygen consumption; HR_{peak}: peak heart rate; CHO: carbohydrate; FFSTM: fat free soft tissue mass; S_I: insulin sensitivity index

In Chapter 3, I explored the high inter-individual variability in CRF response to exercise training in greater depth. In order to achieve this, a median split in ΔVO_{2peak} among the exercise group facilitated a comparison between low and high CRF responders. Accordingly, in this chapter I aimed to compare metabolic rate and substrate utilization at rest and during exercise between low and high CRF responders, as well as differences in body composition, CRF and insulin sensitivity responses to exercise training. I also investigated whether differences in metabolic rate and substrate utilization at baseline might account for a significant proportion of the variability in CRF response to exercise training. High inter-individual variability in CRF response is frequently shown in exercise interventions and is typically attributed to the exercise 'dose' (frequency, intensity and duration of the exercise stimulus)[140,147,150]. In contrast to these studies, low and high responders displayed similar baseline CRF (VO_{2peak}: low responders 25.3 ±2.2 $ml.min^{-1}.kg^{-1}$ versus high responders: 24.5 ±2.7 $ml.min^{-1}.kg^{-1}$) and were exposed to the same exercise dose (average exercise intensity: 78.6 ±4.0 versus 80.6 ±4.0 $\%HR_{peak}$ & and class attendance 38.2 ±6.0 versus 38.6 ±6.9) and yet, by design, CRF in low responders remained largely unchanged while in high responders VO_{2peak} increased by ~21%. High responders showed distinct baseline phenotypic differences to low responders. Compared to low responders, high responders were ~11kg lighter, placing them in a lower BMI category (obese class 1 vs. class 2). During steady state exercise they also derived a greater proportion of energy expenditure from fat oxidation (fat utilization: 62% compared to 41% in low responders), thus exhibiting higher fat oxidation rates and lower carbohydrate oxidation rates compared to low responders. In response to exercise training, high responders lost greater amounts of gynoid fat mass, while in low responders this remained unchanged. These findings further support the results of the previous chapter that highlighted the significance of baseline fat utilization in achieving beneficial changes in body composition in response to exercise training. Using regression models, I further showed that lower baseline rates of carbohydrate oxidation at rest and during steady-state exercise explained 37.5% of the variability in the CRF response to exercise training. Including SAT into the model explained an additional 4.7% of the variability ($p=0.023$), but none of the variables were independent predictors. This is likely because of correlations between baseline SAT along with BMI and steady state RER and fat oxidation rates, such that with increasing SAT, fat utilization and fat oxidation rates declined, a feature that has previously been demonstrated in obese populations with increasing measures of adiposity[154,155]. To the best of my knowledge this is the first study to provide evidence that baseline substrate utilization

influences the subsequent CRF and body composition responses to an exercise intervention. Furthermore, these findings contrast those that have suggested that the exercise dose alone is important in mediating these effects[140,147,150]. Indeed, differences in baseline substrate utilization, particularly among sedentary obese individuals, may in part account for the high inter-individual variability that is increasingly highlighted in outcomes of exercise interventions.

By comparison, despite limited CRF improvements, at post-testing the low responder group improved their ability to oxidize fat and reduced their reliance on carbohydrate oxidation during steady-state exercise. They also increased insulin sensitivity in response to the 12 week exercise intervention. In contrast, insulin sensitivity did not change in high responders despite improvements in CRF and fat oxidation rates at post-testing. Furthermore, for the exercise group as a whole, changes in insulin sensitivity were not associated with changes in CRF, metabolic rate or substrate utilization, at rest or during exercise. At baseline, low responders had lower fat utilization compared to high responders and the subsequent improvements in fat oxidative capacity from this low baseline may have indirectly supported the improvement in insulin sensitivity. In support of these findings, while improvements in insulin sensitivity are frequently shown in exercise intervention studies that induce weight loss[167,176,231,376,377], improved insulin sensitivity in the absence of weight loss tends to be shown only in exercise intervention studies where participants may be starting from a lower baseline fat oxidative capacity such as postmenopausal, overweight and sedentary women[378,379]. By comparison, while calorie restriction has no impact on mitochondrial function, presence of lipid metabolites in skeletal muscle (e.g.., ceramides and diacylglycerol) and plasma inflammatory markers, changes have been observed in the expression of certain proteins involved in insulin-stimulated glucose uptake and utilization in skeletal muscle[380]. This suggests that particular mechanisms may be involved in improved insulin sensitivity through dietary restriction and weight loss specifically. Individuals with higher baseline fat utilization may thus require a more pronounced negative energy balance[381] (achieved through increased exercise EE or the addition of dietary restriction) and in particular significant weight loss[167] in order to show improvements in insulin sensitivity.

Conclusions & Future Directions

In conclusion, in line with my hypothesis, Part 1 showed that exercise training increased CRF and beneficially altered exercise energy expenditure and substrate utilization in sedentary

obese black SA women, a population group within SA shown to be at high risk for increasing obesity, low CRF, insulin resistance and T2DM[86,221,222,382–385]. The very low CRF, typically associated with increased cardio-metabolic risk, was again evidenced in this relatively young group of women, highlighting their increased risk for chronic disease and the need for ongoing exercise interventions to improve CRF[21,224,386]. Baseline substrate utilization was associated with improvements in CRF and body composition. However, neither baseline or change in substrate utilization in response to exercise training were associated with changes in insulin sensitivity. In contrast to non-exercising controls, exercise training prevented weight gain, allowed for small amounts of weight loss and improved anthropometric measures associated with metabolic risk (reduced WC and WHR). Importantly, longitudinal studies in this population group have shown significant gains in body fat measures over time, particularly among younger women, irrespective of habitual activity level[219]. These findings provide further support for the implementation of similar exercise training programs to prevent both weight gain and increasing obesity in this population group shown to be vulnerable to weight gain. However, the exercise intervention failed to achieve clinically significant reductions in body weight required in obesity treatment and did not result in increases in FFSTM which in turn may have impacted on the potential to achieve improvements in S_I[178,179,387].

Weight loss requires the creation of an adequate energy deficit either through dietary restriction or increased EE. Using data collected (RMR, exercise sessions attended, average heart rate of the sessions and results from the VO_{2peak} test) individual regression equations allowed the exercise EE over the duration of the exercise intervention to be estimated for each participant. The mean imposed energy deficit achieved through the exercise intervention was estimated to be ~1,100 calories per week (data not shown), which is more in line with recommendations for improving metabolic health than those required for weight loss[388]. This also compares to ~3,500 calorie per week deficit typically imposed through dietary interventions and more consistently associated with significant weight loss[105,107,108]. Furthermore clinically significant reductions in body weight and improved body composition have been shown in response to exercise-only interventions of similar[111] and longer duration[112] where adherence to exercise prescription (i.e., class attendance) was high (90-100%) and closely controlled. Although I showed no change in dietary intake or physical activity over the duration of the intervention, compensatory responses are also shown to occur in response to an energy deficit even before weight loss occurs[111,116,389]. In order to

improve body weight reductions through exercise interventions an adequate energy deficit is required. This would involve both a progressive increase in the training load as well as greater adherence to exercise prescription. Educating participants around compensatory responses that are shown to occur when an energy deficit is imposed may also help to reduce the widening of an energy gap that could undermine weight loss efforts.

In terms of body composition, it was surprising that VAT, typically improved by exercise training[168], remained unchanged while gynoid FM was preferentially reduced. This response has previously been shown in diet-induced weight loss where African American women lost more abdominal SAT and less VAT compared to white counterparts[390]. Similarly, differences in body fat distribution have been shown in black SA women in comparison to their white counterparts, displaying less central and more peripheral (lower-body) FM as well as less abdominal VAT and more SAT for a given WC [85,237,257]. As such, this novel finding of gynoid FM reduction among exercise participants may describe an ethnic specific, and potentially also a gender specific, body composition response to exercise training.

Interest in understanding inter-individual variability in CRF response to exercise interventions has increased in recent years, but to date these studies have focused predominantly on the exercise dose[140,147,150,277,391]. This thesis presented novel evidence of differences in baseline substrate utilization among sedentary, obese women that explained over a third of the variability in CRF response to exercise training. To date this has not previously been described and potentially contributes to our understanding of inter-individual CRF response variability. Indeed, improvements shown in fat oxidation among low CRF responders may have enhanced their capacity for CRF improvements with ongoing exercise training. This is in line with other studies prescribing a subsequent round of exercise training to eliminate CRF non-response[150]. Similarly, baseline substrate utilization predicted over 60% of the variability in gynoid fat mass reductions, underscoring the role of substrate utilization in body composition improvements in response to exercise training. Inter-individual variability in body composition responses to exercise training have been highlighted in many studies, but without an explanation[142,234]. These findings therefore build on the body of evidence supporting a role for fat oxidation in achieving body composition changes in response to exercise training and highlighting that variability in baseline substrate utilization may predict variability in body composition response. Consequently, individuals with lower capacity for fat oxidation at the start of an exercise program may require a longer

time period before improvements in CRF and body composition are achieved. Identifying individuals that have lower fat oxidative capacity at baseline could allow for a more individualized exercise prescription in order to improve fat oxidation. This may in turn improve the outcomes from exercise interventions such as increased CRF and improvements in body composition.

There were a number of strengths and weaknesses in this study. The pre- and post-testing protocol was well controlled and standardized. Fasting steady state exercise testing allowed for the investigation of energy expenditure and substrate utilization, independent of the acute effects of dietary intake. Each exercise training session was supervised by a trained human movement exercise specialist and objectively monitored using heart rate monitors to ensure adherence to required exercise intensity. The exercise intervention was targeted at black SA women who show the highest risk for obesity and associated risk factors within a SA context. It therefore did not allow for comparisons across other ethic/gender groups. ActivPal data was used as an objective measure of activity during the intervention as the Actigraph data formed part of another PhD student's thesis. This is somewhat unfortunate as it does not allow for a comparable measure to be considered across the two parts of the thesis. Sample size, particularly for the comparison of low and high responders, was relatively small and findings may have been strengthened with increased numbers.

To improve the success of exercise interventions in achieving weight loss, future exercise interventions should aim to increase the exercise training load and adherence to exercise prescription to achieve energy deficits that are more consistent with weight loss. The exercise training modality may also require some refining to improve the potential for gains in FFSTM. A number of findings from the exercise intervention have not previously been shown and more studies are needed to confirm these results. In sedentary obese black SA women, body composition changes in response to exercise training predominantly involved reductions in gynoid FM. This may represent an ethnic/gender specific response and future exercise intervention studies should include additional ethnic/gender comparator groups to investigate this response further. Exercise intervention studies should also be conducted to confirm the relationship between variability in baseline substrate utilization and subsequent changes in both CRF and body composition particularly among sedentary obese populations. This may help to identify responders and non-responders at the outset. Lastly, testing was only carried out at baseline and post-intervention. It was therefore not possible to plot the

time course of changes observed or to identify possible thresholds of substrate utilization that may be associated with improvements in CRF or body composition. Future investigations should therefore include interim testing to allow this to be identified.

PART 2

Summary of findings

In Part 2 of this thesis, I hypothesized that metabolic rate, substrate utilization and insulin sensitivity will be altered through weight loss/regain and may predispose individuals to future weight gain/regain thus impairing successful weight loss maintenance. Figure 6.3 below outlines the compensatory changes shown to accompany weight loss, which may also persist into weight loss maintenance (as detailed in the literature review), together with a summary of the findings from the two studies making up Part 2 of this thesis. In the first study I compared metabolic rate and substrate utilization in the fasted state and in response to a high fat meal challenge, between successfully weight-reduced (lost ≥ 15% body weight, BMI ≤ 27 kg.m^2) and weight-loss relapsed (lost ≥ 15% body weight, regained all weight lost, BMI ≥ 27 kg.m^2) individuals and their BMI-matched counterparts with no weight loss history. In opposition to my hypothesis, I found no evidence of metabolic compensation. Successfully weight-reduced and weight-loss relapsed individuals showed similar metabolic rate and substrate utilization, in both fasted and post-prandial states, compared to respective controls. However, this does not necessarily dispute previous evidence for metabolic compensation in long-term weight-reduced individuals. Instead, I showed that successfully weight-reduced individuals employed lifestyle strategies (diet and physical activity) that were distinct from those of BMI-matched controls which may have counteracted metabolic compensation. Successfully weight-reduced individuals consumed more protein per kilogram, were more physically active and displayed greater FFSTM compared to matched controls. Increased protein intake and physical activity may also have supported higher FFSTM which in turn would support a higher RMR[392,393]. Increased protein intake may also assist with control of appetite and energy intake[394]. Furthermore, weight-reduced individuals showed lower sedentary behaviour and greater moderate and vigorous activity compared to controls, which would counteract compensatory declines in AEE. Nonetheless, subjective reporting of greater dietary restraint, along with the employment of these distinct lifestyle strategies, suggests that maintaining reduced weight remains challenging even after prolonged weight loss maintenance and points to the continued presence of adaptive responses.

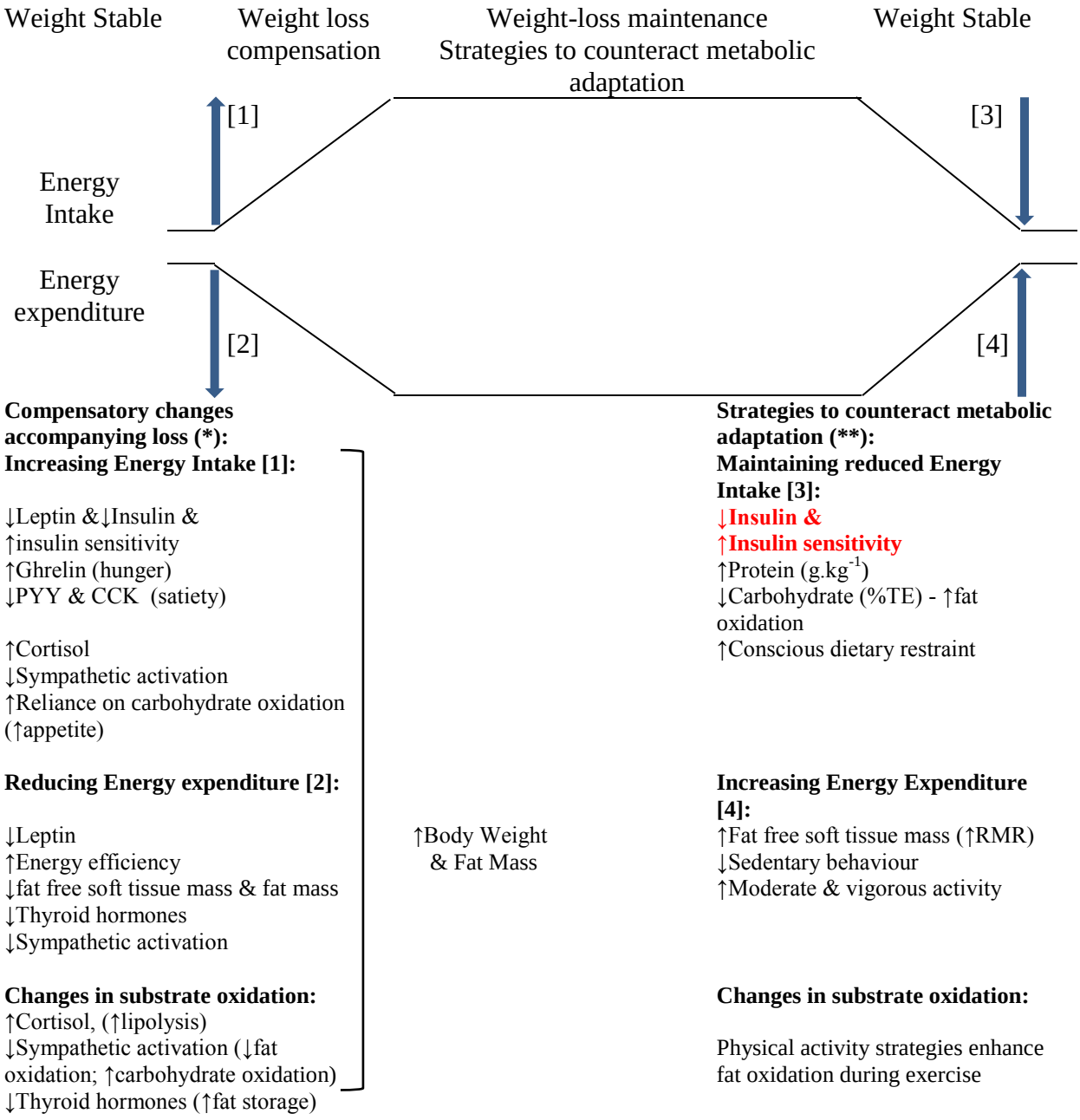


Figure 6.3: Physiological adaption to weight loss and strategies employed in successful weight loss maintenance to counteract the drive to regain body weight. (*) The left hand column details the changes that have been shown to accompany weight loss as highlighted in the literature review and are adapted from several review articles[187,190,197]. (**) The right hand column presents the findings from our studies, highlighting the strategies employed to counteract metabolic adaptation to weight loss and increased S_I among successfully weight reduced compared to all other groups including BMI-matched controls with no weight loss history. Lower plasma insulin levels and greater S_I (highlighted in red) represents an adaptive response to weight loss that has remained present in long-term weight loss maintenance in our study group. Lower insulin levels signal reduced energy stores while greater S_I results in more efficient post prandial nutrient clearance signalling reduced nutrient availability. Together these could lead to increased appetite and may be partly responsible for the need for greater conscious dietary restraint among successfully weight-reduced individuals.

In the final chapter (Chapter 5), I hypothesized that weight-loss would be accompanied by greater insulin sensitivity that would be reversed with weight loss relapse; and that successfully weight-reduced and weight-loss relapsed individuals would display comparable levels of insulin sensitivity relative to phenotypically similar controls with no weight loss history. Insulin sensitivity was assessed using two indices; HOMA-IR (Homeostasis Model Assessment[312]) which uses fasting insulin and glucose measures and is thus indicative of hepatic IR, and the insulin sensitivity index ($ISI_{(0,120)}$, as validated by Gutt *et. al.*, 2000)[359] which uses fasting and 2 hour measures of insulin and glucose, thus representing peripheral insulin sensitivity. In contrast to my hypothesis, my findings showed that successfully weight-reduced individuals were significantly less IR/more insulin sensitive (based on both HOMA-IR and $ISI_{(0,120)}$ measurements respectively) than all other groups, including BMI-matched individuals with no weight loss history. While greater insulin sensitivity is undoubtedly a target objective in obesity treatment, the *enhanced* insulin sensitivity evidenced in significantly weight-reduced individuals may be one of the mechanisms of adaptive response to weight loss that acts to increase risks for weight regain[187]. My results clearly showed that this remains present even after long term maintenance of reduced weight rather than returning to comparable insulin sensitivity levels shown in BMI-matched controls. By contrast, with weight regain these beneficial effects were not observed. In order to explore the variables that were associated with enhanced insulin sensitivity I used 2 regression models (Model 1: predicting log HOMA-IR and Model 2: predicting log $ISI_{(0,120)}$), with both explaining ~60% of the variability in insulin resistance/sensitivity measures. In these models, percentage BW lost and percentage BW regained were the most significant predictors of insulin resistance/sensitivity followed by fat utilization in the fasted state. Previous studies have also found that insulin sensitivity improves following diet-induced weight loss and this was not attributable to changes in specific fat depots, but rather to the effect of weight loss itself[390]. Physical activity, and in particular vigorous activity, was also associated with greater insulin sensitivity (light activity was a significant predictor only in Model 1, thus suggesting an impact on hepatic IR). Measures of body fatness improved the prediction of the models but were not independent predictors. Therefore enhanced insulin sensitivity is evidenced in longer term weight loss maintenance, following significant weight loss, in comparison to phenotypically similar individuals without a history of weight loss and may constitute an ongoing risk for weight loss relapse.

These studies identified individuals who had lost a significant amount of weight and either successfully maintained this weight loss for a median of 30 months or regained all of the weight previously lost. This approach enabled investigation into the longer term effects of weight-loss and regain compared to individuals with no weight loss history. Certainly with comparisons of insulin resistance/sensitivity measures, this was a novel approach to assess the effects of long term weight-loss maintenance. Objective measurements of physical activity also avoided issues of over or under reporting. However, there were limitations. Firstly the studies were cross sectional and therefore, in line with my aims, can only point to associations and make metabolic and behavioural comparisons against matched controls. The studies also relied on self-reported weight history rather than documented weight changes from formal interventions and made use of BIA for measurement of body composition which has limitations. Recruitment of participants for the weight-loss relapsed group and their controls proved to be very difficult and therefore numbers in these groups were relatively lower. Findings, particularly for these groups may have been strengthened had we been able to recruit more participants. As shown in Figure 6.3 above, mechanisms that increase risk for weight loss relapse involve many other hormones besides insulin, including the adipokines such as leptin, appetite hormones released from the gut (e.g. ghrelin, PYY, CCK), thyroid hormones, etc. Unfortunately the current study did not have funding to measure these and this data would have provided a more comprehensive picture of the main physiological drivers that continue to feature in longer term weight loss maintenance. It is imperative to understand these drivers in order to develop effective strategies to counter their effects.

Conclusion

In conclusion to Part 2, although there was no evidence for weight loss associated differences in metabolic rate or substrate utilization among successful weight loss maintainers, they employed distinct strategies from BMI-matched controls that may have counteracted metabolic compensation for weight loss. Together with these lifestyle strategies, they continued to report higher levels of dietary restraint for months and even years (median weight loss maintenance period for weight reduced group was 30 months) following significant weight loss, compared to controls with no weight loss history. This suggests the ongoing presence of adaptive responses to significant weight loss and the need for conscious effort to maintain dietary vigilance and increased physical activity. While these adaptive responses are frequently shown in acute weight loss[181,206], there is less evidence for its presence in long term weight-loss maintenance[208,299,328]. Therefore these findings build on the

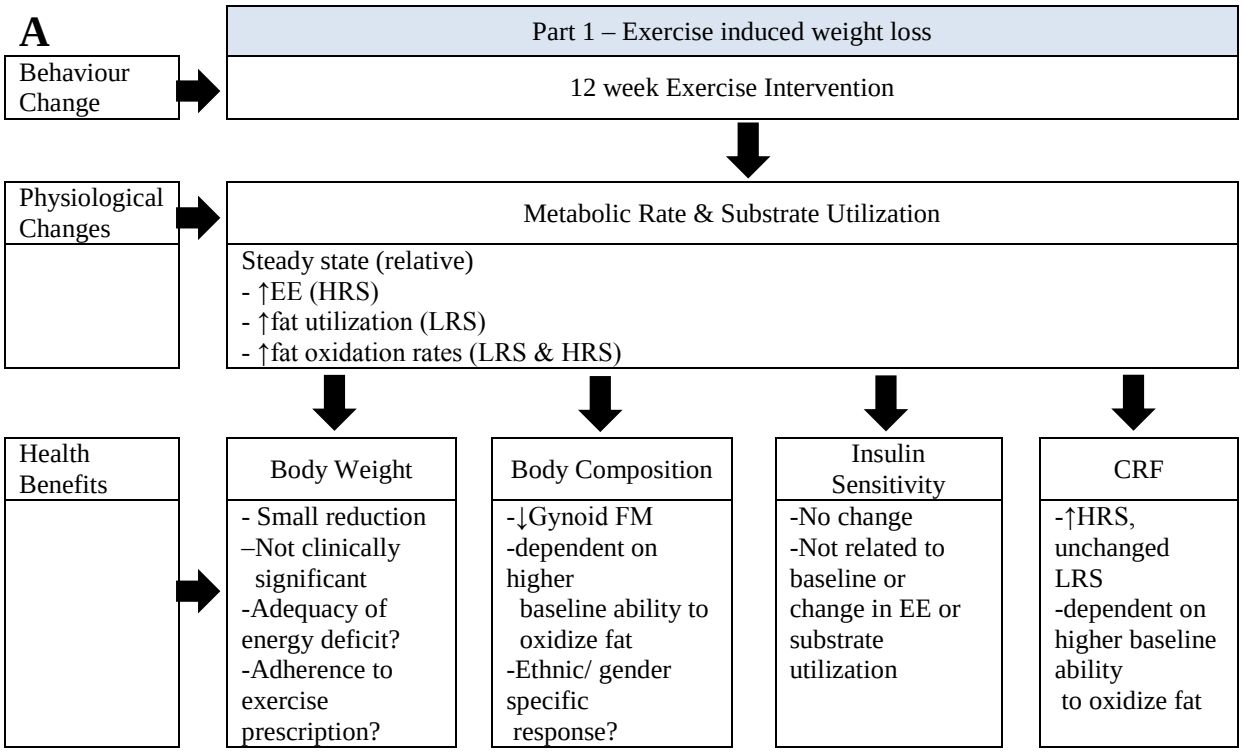
body of evidence in support of longer term persistence of physiological adaption to weight loss and the continued risk for weight loss relapse. It also highlights strategies that may be effective in counteracting these effects. This continuing challenge to maintaining reduced weight, as highlighted in numerous publications[181–184], underscores the need to understand mechanisms of weight loss associated adaptation in obesity treatment. Certainly, both weight loss and weight loss maintenance programs might be improved by incorporating education around physiological adaptation to weight loss, how this might manifest itself behaviourally (increased appetite and EI and reduced EE) while also highlighting the strategies employed by successful weight loss maintainers to counteract this.

Long term, successfully weight reduced individuals demonstrated enhanced S_I. While many studies show improved insulin sensitivity following weight loss, the novelty of this finding lies in the fact that cross-sectional comparison of weight loss associated improvements in insulin sensitivity show that weight reduced individuals are *more* insulin sensitive than BMI-matched controls with no weight loss history. To the best of our knowledge this has not previously been shown. Indeed greater insulin sensitivity is hypothesized to be a contributing mechanism that increases risk for weight loss relapse[187] and may contribute towards the ongoing need for dietary restraint subjectively reported in our successfully weight reduced group. Lower fasting insulin concentrations centrally signal reduced long term body energy stores, while increased insulin sensitivity increases efficiency of postprandial nutrient clearance (i.e. glucose and free fatty acids) thus signalling reduced nutrient availability. Together these tonic and intermittent signals result in an increased appetite and drive for food intake[187]. With a lapse in dietary restraint and reduced physical activity, it is likely that the increased insulin sensitivity, which would affect skeletal muscle, adipose tissue and liver uptake and storage of nutrients[57], could result in rapid weight gain and fat storage. Certainly higher levels of insulin sensitivity are shown to predict increased weight gain[362] and regain following weight loss[395]. Studies have shown the success of dietary macronutrient adjustment to accompany increased, compared to reduced, S_I including a higher carbohydrate component during weight loss[396] and a reduced glycaemic load in weight loss maintenance[364]. When compared to BMI-matched controls, the successfully maintained weight-reduced individuals had indeed made dietary macronutrient adjustments in line with these suggestions, including increased protein (appetite control)[335,397] and reduced carbohydrate intake (reduce dietary glycaemic load). Therefore dietary adjustment, particularly in respect of the insulin-stimulating carbohydrate component, is required not just in the acute period following weight

loss, but also among individuals who have a history of significant weight loss. The ongoing presence of physiological adaption to weight loss, persisting in long term weight loss maintenance, also underscores the need for ongoing cognitive support in maintaining vigilance around dietary intake and physical activity to improve the success of long term maintenance of reduced body weight.

6.2 Overall thesis findings & practical implications

Weight loss is a primary objective in the treatment of obesity and even small amounts of weight loss can reduce associated health risks. The exercise intervention (see Figure 6.4A below), aimed to bring about behavioural change in the form of exercise and to assess physiological adaptions to exercise training that might influence reductions in body weight and improvements in body composition, insulin sensitivity and CRF. Part 2 (see Figure 6.4B) aimed to assess physiological adaptation to long term weight loss maintenance and to explore behavioural strategies adopted by successful weight loss maintainers that may attenuate adaptive physiological responses to weight loss and support weight maintenance. It also compared the beneficial improvement in insulin sensitivity that accompanies weight loss/regain to BMI-matched controls to understand if this returns to similar levels.



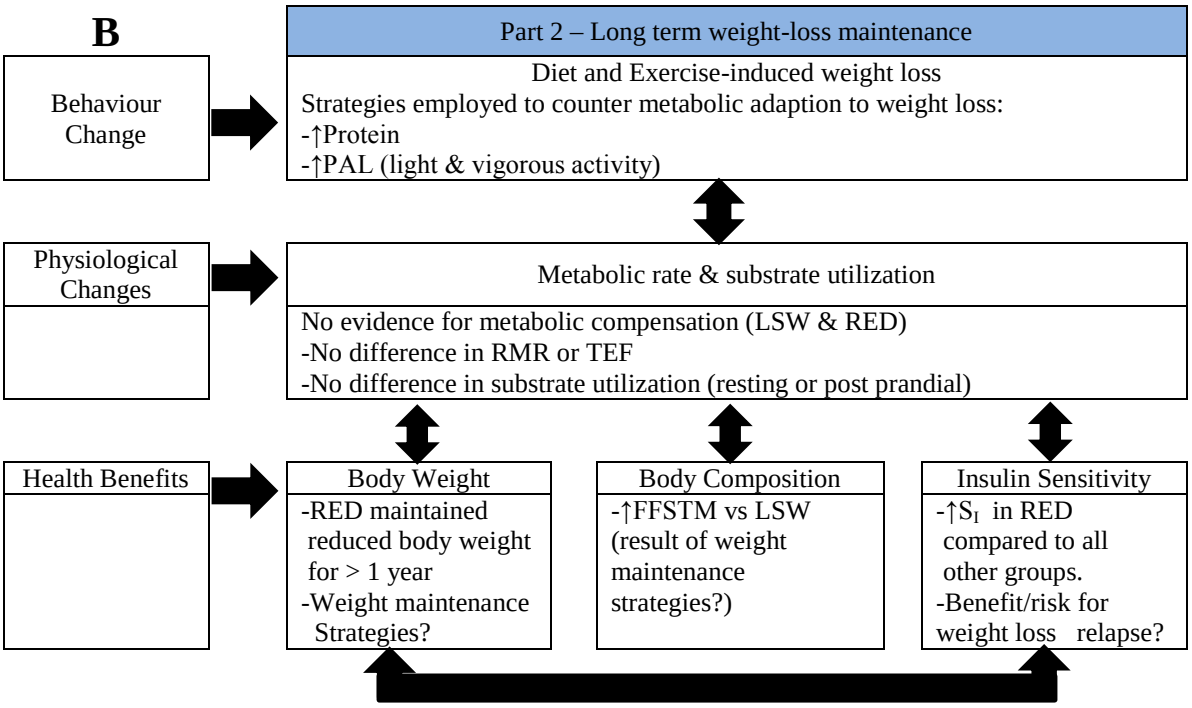


Figure 6.4: The role of metabolic rate and substrate utilization in determining changes in body weight, body composition and insulin sensitivity

Although the exercise intervention prevented weight gain, increased CRF, EE and fat oxidation particularly during submaximal exercise and improved body composition, it did not result in clinically significant weight loss in response to exercise training. Therefore it is likely that the energy deficit, created through increased exercise EE, was of inadequate magnitude to achieve weight loss. Compensatory responses are also likely to have occurred that would act to reduce this energy deficit. If we consider findings from Part 2 of the thesis, several strategies had been employed among successful weight loss maintainers that may have helped to counteract metabolic adaption to weight loss and may in future be used to improve the success of exercise interventions in achieving weight loss. These included maintaining and increasing light activity at the expense of sedentary behaviour, incorporating vigorous activity, maintaining FFSTM (potentially through increased protein intake and physical activity) and maintaining dietary restraint. Educating participants of obesity treatment interventions on the compensatory responses to energy deficit and weight loss (increased EI and reduced spontaneous and planned PAL), together with information on strategies employed by individuals who have achieved and maintained successful weight loss, may provide valuable support and improve outcomes including weight loss.

Nevertheless improvements in body composition and CRF were achieved and therefore the exercise intervention did begin to address aspects of obesity related metabolic risk. These improvements were shown to be related to an individual's baseline capacity to utilize fat to fuel EE both at rest and during exercise. This is important to note as, within this group of phenotypically indistinguishable individuals, despite having similar baseline CRF, participants displayed high variability in baseline substrate utilization that in turn influenced the attainment of outcome measures of the exercise intervention. As whole body fat oxidation is related to underlying mitochondrial volume and oxidative capacity, these differences in baseline characteristics meant that certain individuals were metabolically better placed to achieve improvements in outcome measures in response to exercise training than others. Variability in changes in CRF and body composition are frequently shown in exercise interventions and therefore the ability to identify potential responders and non-responders at baseline would enable individualisation of the exercise stimulus to improve outcomes.

The lack of improvement in insulin sensitivity in response to the exercise intervention is surprising. However, considering findings from Part 2 of this thesis, several factors were shown to predict higher insulin sensitivity, the strongest of which was weight loss. This underscores the importance of achieving a calorie deficit that will facilitate weight loss, particularly in exercise only interventions. Other factors that predicted greater insulin sensitivity in Part 2, included body composition (especially lower measures of body fatness), anthropometric variables (e.g. lower WC and WHR), greater resting fat utilization, higher CRF and increased vigorous physical activity. In the absence of weight loss however, while the exercise intervention did show improvements in some of these measures, this did not translate into improved insulin sensitivity, suggesting that the magnitude of change in response to the exercise training may not have been large enough to impact on insulin sensitivity. Given that the participants were highly sedentary, longer duration exercise interventions may be needed to allow for progressive increases in exercise training load in order to achieve greater improvements in outcome measures including insulin sensitivity. Certainly a dose-response relationship has been observed between exercise undertaken and improvements in outcome variables[377]. It should also be noted that under free living conditions, without incentives, motivation and support the sustainability and ongoing adherence of such exercise interventions may prove challenging. Follow-up focus group discussions may therefore help to highlight potential barriers to exercise adherence and possibly indicate other exercise modalities (e.g. soccer, netball, etc.) that could improve the

ongoing sustainability of such interventions and should be considered in the planning of future studies. With significant weight loss however insulin sensitivity may be improved beyond that shown in BMI-matched controls and is potentially an adaptive response to weight loss. My findings also show that this enhanced insulin sensitivity remains present even after long periods of weight loss maintenance. Weight loss and weight loss maintenance programs should therefore take this into account when considering dietary macronutrient prescription in weight loss and weight loss maintenance to resist the drive to increase EI and regain weight.

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8 Appendix

Supplementary Table 2.1: Associations between CRF, energy expenditure and substrate utilization (resting and steady state exercise) and changes in body composition

	ΔFM		ΔAFM		ΔGFM		ΔSAT		ΔVAT	
	CTL	**EXE**	**CTL**	**EXE**	**CTL**	**EXE**	**CTL**	**EXE**	**CTL**	**EXE**
Cardiorespiratory Fitness										
Baseline VO_{2peak} ($ml.kg^{-1}.min^{-1}$)	0.176	**-0.415**†	0.302	-0.369	0.140	**-0.441**†	0.129	**-0.547**‡	0.446	-0.022
ΔVO_{2peak} ($ml.kg^{-1}.min^{-1}$)	0.395	**-0.466**‡	-0.175	-0.270	0.374	**-0.480**‡	-0.129	-0.172	-0.317	-0.215
Steady State (Baseline)										
EE ($kJ.h^{-1}.\ FFM^{-1}$)	-0.009	-0.371	0.307	-0.258	-0.075	**-0.480**‡	0.345	**-0.437**†	0.194	0.098
RER	-0.249	0.202	0.298	0.051	-0.357	0.413	-0.388	0.055	-0.149	-0.105
Energy derived from Fat (%)	0.213	-0.205	-0.315	-0.053	0.323	**-0.411**†	0.373	-0.061	0.140	0.109
Fat oxidation ($mg.min^{-1}\ .FFM^{-1}$)	-0.261	-0.309	-0.144	-0.145	-0.406	**-0.542**‡	-0.194	-0.220	-0.051	0.137
CHO oxidation ($mg.min^{-1}\ .FFM^{-1}$)	0.206	0.052	0.371	-0.035	0.271	0.214	0.443	-0.087	0.201	-0.071
Steady State (ΔAbsolute)										
ΔEE ($kJ.h^{-1}\ .\ FFM^{-1}$)	0.357	0.344	0.073	0.255	0.311	**0.418**†	-0.142	0.373	0.272	-0.083
ΔRER	-0.304	-0.263	-0.251	-0.266	-0.334	-0.322	-0.321	-0.194	-0.038	-0.114
ΔEnergy derived from Fat (%)	0.346	0.261	0.274	0.257	0.374	0.319	0.342	0.186	0.050	0.100
Δ Fat oxidation ($mg.min^{-1}.\ FFM^{-1}$)	0.405	**0.396**†	0.219	0.357	0.414	**0.481**‡	0.194	0.335	0.136	0.074
Δ CHO oxidation ($mg.min^{-1}.FFM^{-1}$)	-0.202	-0.131	-0.205	-0.171	-0.250	-0.158	-0.346	-0.036	0.054	-0.161
Steady State (ΔRelative)										
ΔEE ($kJ.h^{-1}.FFM^{-1}$)	**0.512**†	-0.111	-0.041	0.015	0.444	-0.106	-0.258	0.097	0.092	-0.063
ΔRER	-0.167	-0.363	-0.257	-0.273	-0.193	**-0.450**‡	-0.281	-0.212	-0.161	-0.078
ΔEnergy derived from Fat (%)	0.188	0.361	0.266	0.266	0.214	**0.446**‡	0.295	0.210	0.157	0.066
Δ Fat oxidation ($mg.min^{-1}\ .FFM^{-1}$)	0.441	0.277	0.201	0.273	0.441	0.352	0.111	0.261	0.194	0.057
Δ CHO oxidation ($mg.min^{-1}\ .FFM^{-1}$)	0.061	**-0.383**†	-0.177	-0.288	0.014	**-0.460**‡	-0.271	-0.212	-0.073	-0.111
Resting (Baseline)										
RMR ($kJ.d^{-1}.FFM^{-1}$)	-0.337	0.284	0.340	0.158	-0.178	0.288	0.451	-0.123	0.105	-0.020
RER	0.009	**0.638**‡	0.419	**0.647**‡	-0.073	**0.562**‡	0.327	**0.662**‡	0.288	0.294
Energy derived from Fat (%)	-0.026	**-0.627**‡	-0.441	**-0.640**‡	0.051	**-0.553**‡	-0.354	**-0.653**‡	-0.290	0.291
Fat oxidation ($mg.min^{-1}.FFM^{-1}$)	-0.276	-0.388	-0.174	**-0.482**‡	-0.092	-0.318	0.000	**-0.542**‡	-0.227	-0.296
CHO oxidation ($mg.min^{-1}.FFM^{-1}$)	-0.099	**0.755**‡	0.447	**0.660**‡	-0.139	**0.680**‡	0.388	**0.650**‡	0.296	0.354
Resting (Δ)										
ΔRMR ($kJ.d^{-1}.FFM^{-1}$)	0.394	-0.067	-0.295	0.070	0.325	-0.078	-0.201	0.020	-0.349	0.195
ΔRER	-0.334	**-0.601**‡	**-0.631**‡	**-0.550**‡	-0.352	**-0.582**‡	**-0.510**†	**-0.572**‡	**-0.504**†	-0.206
ΔEnergy derived from Fat (%)	0.332	**0.590**‡	**0.642**‡	**0.539**‡	0.350	**0.570**‡	**0.526**†	**0.559**‡	**0.498**†	0.200
Δ Fat oxidation ($mg.min^{-1}\ .FFM^{-1}$)	**0.562**‡	0.174	0.423	0.131	**0.478**†	0.131	0.432	0.126	0.062	0.158
Δ CHO oxidation ($mg.min^{-1}\ .FFM^{-1}$)	0.052	**-0.562**‡	**-0.529**†	**-0.444**†	-0.009	**-0.545**‡	**-0.466**†	**-0.491**‡	-0.366	-0.201

BMI: body mass index, WC: waist circumference, FM: fat mass, GFM: gynoid FM, AFM: Android FM, SAT: abdominal subcutaneous adipose tissue, VAT: visceral adipose tissue, FFM: fat free soft tissue mass, $VO_{2peak:}$ peak volume of oxygen consumption, EE: energy expenditure, RER: respiratory exchange ratio, CHO: carbohydrate, RMR: resting metabolic rate . ‡: $p<0.05$, †: $p<0.100$. Note: one participant did not have FFM data and is therefore excluded from analysis involving FFM.

Supplementary Table 3.1 – Comparison of CRF, MR and substrate utilization during Steady State exercise and at rest in low responders and high responders versus the control group

	CTL		LRS		HRS	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Cardiorespiratory Fitness:						
VO_{2peak} (ml.min^{-1})	**2099.4±282^{B}**	**2032.3±196^{AB}**	2,233±100	**2,242±199**	**1,921±173**	**2,314±264***
VO_{2peak} (ml.min^{-1}.kg^{-1})	23.9±3.0	**23.0±2.6$^{A\ B}$**	25.3±2.2	**25.5±2.9**	**24.5±2.7**	**29.7±2.5***
VO_{2peak} (ml.min^{-1}.FFM^{-1})	55.4±8.5	**53.1±7.7$^{A\ B}$**	58.4±3.5	**58.7±3.4**	**55.8±7.2**	**66.3±4.0***
Steady State (Absolute):						
VO_2 (%peak)	52.3±3.7	**51.3±8.1^{B}**	50.6±3.1	47.9±3.1	**51.7±3.1**	**39.9±3.2***
HR (%HRpeak)	65.4±5.5	**64.8±5.5^{B}**	**68.7±3.5**	**62.5±7.0***	**67.6±5.5**	**57.5±6.0***
EE (kJ.h^{-1}.FFM^{-1})	34.3±5.0	32.2±4.6	36.1±3.6	34.0±4.3	34.6±4.7	31.7±4.5
RER	**0.85±0.04^{A}**	**0.85±0.03^{B}**	0.88±0.05	0.85±0.05	0.82±0.03	0.80±0.02
Energy derived from fat (%)	**51.8±14.9$^{A\ B}$**	**51.0±11.0^{B}**	**41.3±16.1**	**50.9±15.4***	61.9±10.4	69.0 ±7.5
Fat oxidation rate (mg.min^{-1}.FFM^{-1})	7.2±2.0	6.7±2.0	6.2±2.6	7.0±2.4	8.7±1.8	8.9±1
CHO oxidation rate (mg.min^{-1}.FFM^{-1})	**18.4±6.4^{A}**	**17.5±3.9^{B}**	**22.9±5.2**	**18.4±6.2***	**14.7±4.4**	**11.1±3.1***
Steady State (Relative):						
VO_2 (%peak)	52.3±3.7	52.7±3.7	50.6±3.1	51.7±2.1	51.7±3.1	51.3±2.1
HR (%HRpeak)	65.4±5.5	65.7±5.5	68.7±3.5	65.7±6.1	67.6±5.5	65.4±4.0
EE (kJ.h^{-1}.FFM^{-1})	34.3±5.0	**33.5±5.4^{B}**	36.1±3.6	36.7±3.8	**34.6±4.7**	**41.0±3.0***
RER	**0.85±0.04^{A}**	**0.86±0.03^{B}**	**0.88±0.5**	**0.85±0.05***	0.82±0.03	0.82±0.03
Energy derived from Fat (%)	**51.8±1.5$^{A\ B}$**	**48.6±9.4^{B}**	**41.3±16.1**	**50.3±16.2***	**61.9±10.4**	**61.8±9.1**
Fat oxidation rate (mg.min^{-1}.FFM^{-1})	7.2±2.0	**6.6±1.8^{B}**	**6.2±2.6**	**7.6±2.7***	**8.7±1.8**	**10.3±1.9***
CHO oxidation rate (mg.min^{-1}.FFM^{-1})	**18.4±.4^{A}**	19.0±4.6	**22.9±5.2**	19.8±6.1	14.7±4.4	17.5±4.0
Speed (km.h^{-1})	3.0±0.1	3.0±0.0	3±0	3±0	3±0	3±0
Gradient (%)	4.0±3.1	**4.3±2.3$^{A\ B}$**	**4.0±2.5**	**7.8±4.0***	**3.6±.8**	**10.6±.2***
Resting Measurements:						
RMR (kJ .FFM^{-1})	150±19	161±20	156±23	159±26	135±31	153±30
RER	0.79±0.04	**0.77±0.05^{B}**	**0.81±0.03**	**0.78±0.04***	**0.79±0.05**	**0.82±0.04***
Energy derived from fat (%)	71.8±12.9	**77.1±16.4^{B}**	**63.1±10.8**	**75.4±12.8***	**73.1±18.2**	**62.0±12.3***
Fat oxidation rate (mg.min^{-1}.FFM^{-1})	1.8±0.4	**2.0±0.5^{B}**	**1.62±0.45**	**2.07±0.56***	1.67±0.60	**1.52±0.35**
CHO oxidation rate (mg.min^{-1}.FFM^{-1})	1.9±1.0	**1.6±0.9^{B}**	2.6±0.9	1.8±1.0	**1.8±0.8**	**2.6±1.4***

NOTE: VO_{2peak}: peak volume of oxygen consumption; VO_2: volume of oxygen consumption; HR: heart rate; $HR\%_{peak}$: heart rate as a percentage of peak HR;EE: energy expenditure; RER: respiratory exchange ratio; CHO: carbohydrate; RMR: resting metabolic rate. *: indicates within group change over time ($p<0.05$). Superscript ^{A}: Group difference CTL versus LRS at pre or post testing ($p<0.05$); superscript^{B}: Group difference CTL versus HRS at pre post testing ($p<0.05$).

Supplementary Table 3.2 – Comparison of body composition and insulin sensitivity in low and high responders versus the control group

	CTL		LRS		HRS	
	Pre	Post	Pre	Post	Pre	Post
Body Composition:						
Weight (kg)	**87.8±10.9^{B}**	**88.8±11.0^{B} ***	89.5±6.0	88.4±7.8	**78.7±7.7**	**78.2±8.9**
BMI (kg.m^{-2})	**33.4±2.7^{A}**	**33.8±2.8***	**36.2 (35.3-37.1)**	35.9 (34.8-37.5)	31.8 (30.6-33.9)	31.0 (30.0-34.5)
WC (cm)	**103.4±8.1**	**105.9±9.5^{B} ***	**108 (100-109)**	**102* (95-109)**	**100 (98-105)**	**97* (94-103)**
HC (cm)	**117.5±1.6^{B}**	**117.9±1.6^{B}**	118 (114-123)	117 (112-123)	**113 (105-116)**	**110* (104-113)**
WHR	0.88±0.05	0.90±0.07	0.89 (0.87-0.93)	0.87 (0.86-0.90)	0.90 (0.87-0.94)	0.89 (0.86-0.91)
FFSTM (kg)	**38.4^{B} (35.0-40.7)**	**38.2^{B} (35.4-38.2)**	39.0 (36.8-40.3)	39.3 (35.9-40.1)	**34.3 (32.7-37.4)**	**35.3 (33.0-37.2)**
BF%	50.4±4.3	50.4±3.9	49.9 (48.7-51.7)	50.2 (49.3-52.8)	50.0 (48.3-51.6)	48.8 (48.1-50.4)
FM (kg)	**40.8±7.0^{B}**	**41.2±6.2^{B}**	41.2±5.1	42.0±6.5	**36.1±4.9**	**35.3±5.3**
Trunk (%FM)	45.8±4.7	45.6±4.7	49.6±3.4	50.8±4.5	47.8±3.2	46.8±3.0
Android (%FM)	3.3±1.0	3.3±1.0	8.2±.8	8.1±0.9	8.3±1.3	8.2±1.2
Gynoid (%FM)	19.4±2.3	**19.6±2.3^{B}**	18.7±1.8	18.6±1.5	**18.2±1.7**	**17.8±1.6***
SAT (cm^{2})	533±100	533±09	566±75	565±81	491±54	482±60
VAT (cm^{2})	128.2 (87.4-168.0)	136.7 (92.8-157.8)	131.1 (121.9-165.0)	140.9 (119.1-152.4)	125.2 (121.9-163.8)	129.1 (121.6-136.3)
Insulin Sensitivity:						
FPG (mmol.L^{-1})	5.02±0.66	5.08±0.79	5.6 ±1.0	5.0 ±.0	5.4 ±0.7	5.2 ±1.0
F-Ins (mU.L^{-1})	13.0 (9.6-14.2)	12.9 (7.7-19.6)	15.5 (14.2-19.0)	13.0 (12.3-16.3)	12.2 (6.4-19.9)	11.2 (10.5-17.1)
HOMA2%IR	1.8 (1.4-2.2)	2.0 (1.1-2.9)	2.4 (2.1-2.7)	2.0 (1.6-2.3)	1.7 (1.0-2.9)	1.7 (1.4-2.6)
S_I	2.5 (1.3-4.1)	2.0 (1.7-3.0)	**2.1 (1.3-3.1)**	**2.3 * (1.5-4.7)**	1.7 (1.2-2.4)	2.0 (1.5-3.1)

*: indicates within group change over time ($p<0.05$). Superscript ^{A}: Group difference CTL versus LRS at pre or post testing ($p<0.05$); superscript^{B}: Group difference CTL versus HRS at pre post testing ($p<0.05$).

Supplementary Table 3.3 – Energy Intake, Macronutrient Distribution and Daily Step Count in controls, low responders and high responders

	CTL	LRS	HRS
EI (kJ)			
Baseline	8,138 (6,493-9,434)	9,633±4,118	8,602±2,075
Week 4	7,489 (7,110-9,217)	7,786±2,210	8,479±1,911
Week 8	7,963 (6,306-8,921)	9,008±2,827	8,522±1,376
Week 12	8,429 (7,335-9,509)	8,572±1,752	8,657±1,673
EI:RMR (ratio)			
Baseline	1.458 (1.250-1.865)	1.426 (1.222-1.973)	1.821 (1.568-2.265)
Post-testing	1.499 (1.104-1.693)	1.521 (0.968-1.902)	1.859 (1.292-2.191)
Protein (%EI)			
Baseline	14.3±1.9	13.2±2.8	13.3±2.4
Week 4	13.2±2.3	14.0±3.0	13.8±3.0
Week 8	13.8±2.8	13.4±1.9	13.4±2.2
Week 12	13.5±2.1	14.3±3.6	13.2±2.5
Carbohydrate (%EI)			
Baseline	54.0±5.7	55.5±6.5	54.6±4.5
Week 4	56.9±6.0	55.6±5.1	52.2±6.4
Week 8	56.0±7.6	55.4±5.0	53.1±5.6
Week 12	**57.8±7.2*** [B]	53.0±6.4	**52.4±10.2**
Fat (%EI)			
Baseline	31.0±5.6	30.3±7.8	30.6±4.0
Week 4	29.2±5.1	29.5±5.5	32.5±6.4
Week 8	29.5±7.3	30.2±5.2	32.4±5.8
Week 12	**27.8±6.4***	32.1±3.7	32.3±7.7
Daily step count			
Baseline	10,082±2,598	9,118±2,688	9,843±1,846
Week 4	**9,055±2,471**[B]	10,436±2,498	**11,827±2,031**
Week 8	**8,784±2,785**[B]	10,368±3,104	**10,955±4,035**
Week 12	**9,242±1,791**[A B]	**11,666±3,052***	**11,587±4,077***

*: indicates within group change over time ($p<0.05$). Superscript [A]: Group difference CTL versus LRS at pre or post testing ($p<0.05$); superscript[B]: Group difference CTL versus HRS at pre post testing ($p<0.05$). The EI:RMR ratio was used to determine the degree of under reporting of dietary energy intake using a cut-off of <1.35 as defined by Goldberg et al (1991)[261]. Using this cut-off, under reporting of EI at pre-testing: 6/15 of CTL, 4/10 of LRS and 0/10 of HRS and at post-testing: 6/15 of CTL, 4/10 of LRS and 4/10 of HRS were identified.

MAXIMAL GRADED EXERISE TEST

Name: _____ Code:_____ Date/ Time: _____

Age:_____ Weight: _____ Height: _____

Last menstrual period:_____

Last exercise session:_____ Type and intensity:_____

Time of last meal/drink: _____ Hours fasted:_____

Meal:_____

How would you describe your health TODAY? (How are you feeling?):

Good ☐ Fair ☐ Poor ☐

If POOR, explain why:_____

Age-predicted max heart rate_____bpm

Check List before starting (tick each box once done):

RPE explanation	Familiarization	Gradient test	Side straddle	Emergency stop

HRM ON ☐

Time	Speed	Gradient	Heart Rate	RPE	RER	VO2/kg
1 min	3.0km/h	2%				
2 min	3.0km/h	2%				
3 min	3.0km/h	4%				
4 min	3.0km/h	4%				
5 min	3.0 km/h	6%				
6 min	3.0 km/h	6%				
7 min	3.0 km/h	10%				
8 min	3.0 km/h	14%				
9 min	3.0 km/h	16%				
10 min	3.5 km/h	16%				
11 min	3.5 km/h	17%				
12 min	4.0 km/h	18%				
13 min	4.5 km/h	18%				
14 min	5.0km/h	18%				
15 min	5.0km/h	19%				
16 min	5.0km/h	20%				
17 min	5.0km/h	21%				
18 min	5.0km/h	22%				
19 min	5.km/h	23%				

TEST Terminated: _____ **HRM STOP** ☐ **HRM BELT OFF:** ☐

VO_{2max} _____ 50% VO_{2max} _____

RESTING METABOLIC TEST

Name: _____ Code:_____

Weight: _____ Height:_____ Time:_____

Last menstrual period:_____

Last exercise session:_____ Type and intensity:_____

Time of last meal/drink: _____ Hours fasted: _____

Meal:_____

How would you describe your health TODAY? (How are you feeling?):

☐ Good ☐ Fair ☐ Poor

If POOR, explain why:

_____

RESTING

Time	Heart Rate	RER	Comments
0 min			
5 min			
10 min			
15 min			
20 min			
25 min			
30 min			
35 min			
40 min			
45 min			

Resting heart rate (lowest heart rate recorded):_____

STEADY STATE EXERCISE

Name: _____ Code:_____ Time:_____

VO_{2max} _____ HR @ VO_{2max} _____

50% VO_{2max} _____ HR @ 50% VO_{2max} _____

Speed:_____

Gradient:_____

Check List before starting (tick each box once done):

RPE explanation	Familiarization	Gradient test	Side straddle	Emergency stop

Speed	**Gradient**	**Time**	**HR**	**RPE**	**RER**	**VO2**
		1 min				
		2 min				
		3 min				
		4 min				
		5 min				
		6 min				
		7 min				
		8 min				
		9 min				
		10 min				
		11 min				
		12 min				
		13 min				
		14 min				
		15 min				
		16 min				
		17 min				
		18 min				
		19 min				
		20 min				

Comments:_____

Questionnaires: Chapters 4 & 5 – Mind the Gap Study

MEDICAL HISTORY: FAMILY

Where applicable, underline which of your FIRST DEGREE RELATIVES (mother/father/sister/brother) have presented with the following:

1. Stroke (mother/father/sister/brother) Year
2. Diabetes (mother/father/sister/brother) Year
3. Obesity (mother/father/sister/brother) Year
4. High cholesterol (mother/father/sister/brother) Year
5. High blood pressure (mother/father/sister/brother) Year
6. Cancer (mother/father/sister/brother) Year
7. Heart attack (mother/father/sister/brother) Year
8. Other heart conditions (mother/father/sister/brother) Year
9. Angina (heart pains) (mother/father/sister/brother) Year
10. Bypass surgery (mother/father/sister/brother) Year
11. Peripheral cardiovascular disease (of the veins in the arms and legs) (mother/father/sister/brother) Year
12. Coronary artery disease (mother/father/sister/brother) Year
13. Sudden death under the age of 60 (due to natural causes) (mother/father/sister/brother) Year
14. Other conditions not mentioned (mother/father/sister/brother) Year

MEDICAL HISTORY: SELF

Please circle yes or no to indicate whether YOU have suffered from the following:

1. Stroke: No Yes Year
2. Diabetes: No Yes Year

3. Obesity: No Yes Year
4. High cholesterol: No Yes Year
5. High blood pressure: No Yes Year
6. Cancer: No Yes Year
7. Heart attack: No Yes Year
8. Other heart conditions: No Yes Year
9. Angina (heart pains): No Yes Year
10. Bypass surgery: No Yes Year
11. Peripheral cardiovascular disease (of the veins in the arms and legs): No Yes Year
12. Coronary artery disease: No Yes Year
13. Other conditions not mentioned: No Yes Year

REPRODUCTIVE HISTORY:

What was your age when you had your first period: years old

Please select the average time interval between your cycles:

1. 25-30 days 2. 30-35 days 3. 36 days or more

Please circle (+/-) the day of your cycle you will be on [on 1st DAY OF TESTING]:
1 2 3 4 5 6 7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22 23 24 25 26 27 28
[Day 1 = day of bleeding]

Have you ever had a hysterectomy?

0. No 1. Yes

Have you reached menopause?

0. No 1. Yes

Number of pregnancies:

0. None 1. One 2. Two 3. Three 4. Four 5. Five 6. Six or more

Number of children breastfed:

0. None 1. One 2. Two 3. Three 4. Four 5. Five 6. Six or more

Are you lactating (i.e. are your breast producing milk)?

0. No 1. Yes

Are you using contraception?

0. None 1. Oral 2. Injectable 3. Condoms 4. Intrauterine Device 5. Other

For how long have you been using these contraceptives? years

Have you used other forms of contraception in the past?

0. None 1. Oral 2. Injectable 3. Condoms 4. Intrauterine Device 5. Other

For how long did you use these other forms of contraception? years

GENERAL HEALTH HABITS:

Do you smoke?

0. No, I don't smoke and have never smoked before
1. I quit smoking less than 6 months ago
2. I quit smoking more than 6 months ago
3. I smoke 1-5 cigarettes per day
4. I smoke 6-10 cigarettes per day
5. I smoke 11-15 cigarettes per day
6. I smoke one pack (20 cigarettes) per day or more

On average, how many units of alcohol do you drink on a WEEK DAY? 0. 0

1. 1
2. 2
3. 3
4. 4
5. 5 or more

On average, how many units of alcohol do you drink on a WEEKEND DAY? 0. 0

1. 1
2. 2
3. 3
4. 4
5. 5 or more

On average, how many times per week do you drink more than 6 drinks in 1 sitting? 0. 0

1. 1
2. 2
3. 3
4. 4
5. 5 or more

On average, how many times per week do you eat double as much as you think you should in 1 sitting, or eat when you are not hungry?

0. 0
1. 1
2. 2
3. 3
4. 4
5. 5 or more

How would you rate your stress level at HOME?

0. Little/no stress
1. Below average stress level
2. Average stress level
3. Above average stress level
4. Highly stressful

How would you rate your stress level at WORK?

0. Little/no stress
1. Below average stress level
2. Average stress level
3. Above average stress level
4. Highly stressful

Are you currently taking anti-depressant medications?

0. No
1. Yes

BASIC DEMOGRAPHIC INFORMATION:

Please identify your marital status:

1. Single
2. Married
3. Divorced
4. Widowed

Please indicate your highest level of education achieved:

1. High school
2. Matriculation
3. College/tech.
4. Undergraduate degree
5. Postgraduate degree

Please identify your occupation:

What is the nature of your work?

1. Unemployed
2. Agriculture
3. Mining/quarrying

4. Manufacturing
5. Construction
6. Transport
7. Trade
8. Services
9. Education
10. Health
11. Administration
12. Management
13. Student

Please select your monthly income bracket:

1. No income
2. R0001-R2499
3. R2500-R4999
4. R5000-R7499
5. R7500-R9999
6. R10 000 or more

Do you receive supplementary financial support or funding?

0. No additional income
1. Government
2. Spouse
3. Friend

4. Family

Which of these best describes your living arrangements?

1. Studio apartment
2. 1 bedroom dwelling
3. 2 bedroom dwelling
4. 3 bedroom dwelling
5. 4 bedroom dwelling
6. 5 or more bedroom dwelling

How many housemates or flatmates do you have? 0. 0

1. 1
2. 2
3. 3
4. 4
5. 5 or more

Do you own a car?

0. No car
1. 1 car
2. 2 cars
3. 3 or more cars

Do you own a cell phone?

0. No

1. Yes

THREE FACTOR EATING QUESTIONNAIRE

Question 1: When I smell a sizzling steak or see a juicy piece of meat, I find it difficult to keep from eating, even if I've just finished a meal.

0) False

1) True

Question 2: I usually eat too much at social occasions, e.g. parties.

0) False

1) True

Question 3: I am usually so hungry that I eat more than 3 times a day.

0) False

1) True

Question 4: When I have eaten my quota of calories, I am usually good about not eating any more.

0) False

1) True

Question 5: Dieting is so hard for me because I just get too hungry.

0) False

1) True

Question 6: I deliberately take small helpings so as to control my weight.

0) False

1) True

Question 7: Sometimes things just taste so good that I keep on eating, even when I am no longer hungry.

0) False

1) True

Question 8: Since I am often hungry, I sometimes wish that an expert would tell me when I have had enough or when I can still have something more to eat.

0) False

1) True

Question 9: When I feel anxious, I find myself eating.

0) False

1) True

Question 10: Life is too short to worry about dieting.

0) False

1) True

Question 11: Since my weight goes up and down, I have gone on reducing diets more than once.

0) False

1) True

Question 12: I often feel so hungry that I just have to eat something.

0) False

1) True

Question 13: When I am with someone who overeats, I usually overeat too.

0) False

1) True

Question 14: I have a pretty good idea of the number of calories in foods.

0) False

1) True

Question 15: Sometimes when I start eating, I just can't seem to stop.

0) False

1) True

Question 16: It is not difficult for me to leave something on my plate.

0) False

1) True

Question 17: At certain times of the day, I get hungry because I have gotten used to eating at these times.

0) False

1) True

Question 18: While on a diet, if I eat a food that is not allowed, I consciously eat less for a period of time to make up for it.

0) False

1) True

Question 19: Being with someone who is eating often makes me hungry enough to eat too.

0) False

1) True

Question 20: When I feel blue/down, I often overeat.

0) False

1) True

Question 21: I enjoy eating too much to spoil it by counting calories or watching my weight.

0) False

1) True

Question 22: When I see a real delicacy, I often get so hungry that I have to eat it right away.

0) False

1) True

Question 23: I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.

0) False

1) True

Question 24: I get so hungry that my stomach often seems like a bottomless pit that cannot be

filled.

0) False

1) True

Question 25: My weight has hardly changed at all over the last 10 years.

0) False

1) True

Question 26: I am always hungry, so it is hard for me to stop eating before I finish the food on my plate.

0) False

1) True

Question 27: When I feel lonely, I console myself with food.

0) False

1) True

Question 28: I consciously hold back at meals in order to avoid weight gain.

0) False

1) True

Question 29: I sometimes get very hungry late in the evening or at night.

0) False

1) True

Question 30: I eat anything I want, any time I want.

0) False

1) True

Question 31: Without even thinking about it, I take a long time to eat.

0) False

1) True

Question 32: I count calories as a conscious means of weight control.

0) False

1) True

Question 33: I do not eat certain foods because they make me fat.

0) False

1) True

Question 34: I am always hungry enough to eat at any time.

0) False

1) True

Question 35: I pay a great deal of attention to changes in my figure.

0) False

1) True

Question 36: While on a diet, if I eat a food that is not allowed, I often splurge and over eat high calorie foods.

0) False

1) True

Question 37: How often do you diet as a conscious weight control method?

1) Rarely

2) Sometimes

3) Usually

4) Always

Question 38: Would a weight fluctuation of 2.5kg affect the way you live your life?

1) Not at all

2) Slightly

3) Moderately

4) Very much

Question 39: How often do you feel hungry?

1) Only at meal times

2) Sometimes between meals

3) Often between meals

4) Almost always

Question 40: Do your feelings of guilt about overeating help you to control your food intake?

1) Never
2) Rarely
3) Often
4) Always

Question 41: How difficult would it be for you to stop eating halfway through dinner and not eat for the next 4 hours?

1) Easy
2) Slightly difficult
3) Moderately difficult
4) Very difficult

Question 42: How conscious are you of what you eat?

1) Not at all
2) Slightly
3) Moderately
4) Extremely

Question 43: How often do you avoid “stocking up” on tempting foods?

1) Almost never
2) Seldom
3) Usually
4) Almost always

Question 44: How likely are you to shop for low calorie foods?

1) Unlikely
2) Slightly likely
3) Moderately likely
4) Very likely

Question 45: Do you eat sensibly in front of others and splurge alone?

1) Never
2) Rarely
3) Often
4) Always

Question 46: How likely are you to consciously eat slowly in order to cut down on how much you eat?

1) Unlikely
2) Slightly likely
3) Moderately likely
4) Very likely

Question 47: How frequently do you skip dessert because you are no longer hungry?

1) Almost never
2) Seldom
3) At least once a week
4) Almost every day

Question 48: How likely are you to consciously eat less than you want?

1) Unlikely
2) Slightly likely
3) Moderately likely
4) Very likely

Question 49: Do you go on eating binges even though you are not hungry?

1) Never
2) Rarely
3) Sometimes
4) At least once a week

Question 50: How well does this statement describe you: “I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.”

1) Not like me
2) A little like me
3) Pretty good description of me
4) Describes me perfectly

Question 51: Where would you place yourself on the following scale? You:

1) Eat whatever you want, whenever you want it
2) Usually eat whatever you want, whenever you want it
3) Often eat whatever you want, whenever you want it
4) Often limit food intake, but often “give in”
5) Often limit food intake, rarely “give in”
6) Constantly limit food intake, never “giving in”