The effects of the 5:2 intermittent fasting diet and sprint interval training on body composition and markers of cardiovascular and metabolic health in overweight individuals

by

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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January 2020

Abstract

The global prevalence of obesity has increased almost threefold in recent decades, predicated on the increased availability of energy dense foods, decreased physical activity and the increasingly sedentary nature of the modern workforce.

The 5:2 intermittent fasting diet (IF) and sprint interval training (SIT) are two time efficient and easy to follow strategies that have recently become popular alternatives to CR and MICT strategies for weight loss. The efficacy of IF and SIT on weight measurement and health outcomes have been demonstrated separately but the combined effects of these protocols (IFSIT) are currently unknown. Therefore, the purpose of this thesis is to investigate the effects of these protocols (individually and in combination) on body composition, as well as cardiovascular and metabolic risk factors associated with obesity in a free-living, adult population. Additionally, this thesis will investigate the effects of these protocols on mood state, satiety and activity levels to further understand the wider effects of these strategies on motivation and wider health.

Thirty-four participants were randomised into three groups (fasting only, SIT only & a combined protocol). The 5:2 protocol uses 2 non-consecutive days of severe energy restriction interspersed with 5 days of *ad libitum* food consumption per week. The SIT protocol used in the current study used 20 seconds of supramaximal exercise (150% VO_{2max}) interspersed with 40 seconds of active rest over 3-6 cycles. VO_2 testing was carried out using a ramped load program on an electrically braked cycle ergometer. Body composition was assessed using DEXA and pqCT and blood analysis was performed following an overnight fast and analysed either commercially or using enzyme-linked immunosorbent assay.

The findings of this thesis demonstrate that following a 16-week intervention in an unrestricted, free-living population that IF, with or without SIT, has reduced body weight (IF= -4.1 kg, IFSIT= -4.5 kg), BMI (IF= -1.4, IFSIT= -1.6) and fat mass (IF= -3.3 kg, IFSIT= -2.9 kg) , whilst SIT alone had little effect on these parameters despite significant effects being reported in other studies. Interestingly, IFSIT led to a greater loss of lean mass when compared to IF alone (IF= -0.75 kg, IFSIT= -1.8 kg). Additionally, significant increases to VO_{2peak} (ml/kg.minute) (SIT= +2.1, IFSIT= +4.6) in both exercise groups were observed. However, there was little improvement to other cardiovascular risk factors such as arterial compliance, blood pressure measurements or lipid profiles.

Significant decreases in serum leptin levels were recorded in both the IF and IFSIT groups when compared to the SIT group, with little change in other parameters such as glucose tolerance, fasting glucose, HBA1c and other metabolic hormone levels. Additionally, there was no significant effects on mood state, satiety, quality of life measurements, attitudes to food or total activity levels recorded in any group.

In conclusion, this thesis is one of the first to demonstrate that the 5:2 diet is effective in the reduction of body weight, leptin levels, BMI and fat mass in a free-living population, whilst SIT had no impact on whole body adiposity despite improvements to VO_{2peak} measurements. When combined with the 5:2 diet, sprint interval training led to greater reductions in lean muscle mass when compared IF alone but otherwise produced no further additive effects. These data suggest that the 5:2 diet is an effective and safe alternative to more traditional energy restriction diets for weight loss in free living adult population.

Declaration of Authenticity

I, William Deasy, declare that the PhD thesis entitled “The effects of the 5:2 intermittent fasting diet and sprint interval training on body composition and markers of cardiovascular and metabolic health in overweight individuals” is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Signature  Date: 10/01/2020

ACKNOWLEDGEMENTS

It has been a busy few years, with many problems and hurdles overcome along the way, but it has also been a lot of fun. This project would have been impossible without the help and assistance of many people along the way. I would particularly like to thank the following people:

Thank you to Dr Christos Stathis and Dr Matt Cooke, my supervisors, for giving me the opportunity to undertake this PhD under their supervision and for their guidance, patience and perpetual optimism along the way.

A massive thank you to Robin Wilson, for his help with participant training and testing. You made a hard job a lot more fun and manageable!

Thank you to Ben Harvey and Jessica Danaher for providing help in learning the techniques and protocols that I needed for this project.

Many thanks to Dr Ebrahim Bani Hassan for his assistance with analysis of pqCT scans.

Many thanks to my Wife, Cherelle, for her endless support and for bank rolling part of this whole enterprise.

And finally, to my friends and family for their love, support and patience.

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
ADF	Alternate Day Fasting
AMP	Adenosine Monophosphate
ATSI	Aboriginal and Torres Strait Islander
AUC	Area under curve
BDNF	Brain Derived Neurotrophic factor
BMI	Body Mass Index
CAD	Coronary Artery Disease
CBP	Central Blood Pressure
CR	Calorie Restriction
CVD	Cardiovascular disease
DEXA	Dual Energy X-ray Absorptiometry
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ER	Energy restriction
Flox	Sodium fluoride/Potassium oxalate
FMD	Fasting Mimicking Diet
HBA1c	Glycated Haemoglobin/Average Plasma Glucose
HDL	High Density Lipoprotein
HOMA2-IR	Homeostatic model assessment of insulin resistance
HR	Heart Rate
HWR	Hip to waist ratio
IER	Intermittent Energy Restriction
IF	intermittent Fasting
IGF-1	Insulin-like Growth Factor 1

IL-6	Interleukin 6
IMAT	Intramuscular Adipose Tissue
INS-7	Insulin-like Protein 7
Kcal	Kilocalories
LDL	Low Density Lipoprotein
MDD	Major Depressive Disorder
MICT	Moderate Intensity Continuous Training
*m*ADF	Modified Alternate Day Fasting
MPS	Muscle Protein Synthesis
OD	Optical Density
PARs	Predictive Adaptive Responses
PBP	Peripheral Blood Pressure
PD	Parkinson's Disease
PF	Periodic Fasting
pqCT	Peripheral Quantitative Computed Tomography
PWA	Pulse wave analysis
PWV	Pulse wave velocity
RER	Respiratory exchange ratio
RIF	Ramadan Intermittent Fast
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RPE	Rating of perceived exertion
S.E.M.	Standard Error Mean
SIRT1	Sirtuin 1
SIT	Sprint interval training
TAG	Triacylglyceride
TG	Triglyceride

TNF-α	Tumour necrosis factor alpha
VHDL	Very High Density Lipoprotein
VO_{2max}	Maximum aerobic capacity
VO_{2peak}	Peak aerobic capacity

Chapter 1 – General Introduction

Since the industrial revolution, society has undergone numerous shifts, with alterations to sleeping patterns, activity levels and eating habits associated with expanding urbanisation, light pollution, decreased physical activity and the ready availability of food (Malik *et al.*, 2012). These factors have led to a marked increase in obesity rates and over the last few decades obesity, metabolic syndrome and Type 2 diabetes have reached unprecedented levels (WHO, 2010; WHO, 2018). Concomitantly, spiralling increases in the costs associated with treating these conditions and their related co-morbidities (particularly given the earlier onset of this weight gain) have created a multifaceted pandemic. The associated economic burden of healthcare has driven interest in finding effective, safe and easy to follow lifestyle-based interventions that can be applied to a wide range of people within the community (Malik et al., 2012; Imes & Burke, 2014; Ng *et al.*, 2014; Harvie & Howell, 2017).

Traditional strategies have emphasised the use of long-term energy restriction (typically 15 - 40% of RDI) in combination with moderate intensity continuous exercise in a direct attempt to address the root cause of lifestyle sustained positive energy balance caused by reduced activity levels and a hypercaloric dietary intake (Del Corral *et al.*, 2009; Varady & Hellerstein, 2008). Unfortunately, this approach doesn't account for the metabolic and hormonal drivers of overeating and, while effective if followed correctly, is often subject to poor compliance as patients often find the constant energy restriction, calorie counting and exercise onerous (Del Corral *et al.*, 2009). This is a problem exacerbated by the varied working hours and extended travel times that are a common feature of the modern lifestyle. These factors, in combination with family and social commitments, often leave individuals time-poor and unwilling, or unable to allocate the time required to maintain their health (Dishman *et al.*, 1985; Sabinsky *et al.*, 2007).

The search for solutions to reverse the tide of increasing obesity has led to more widespread use of bariatric surgeries such as lap-band and gastric sleeving as a solution for the correction of obesity. While effective, these procedures have all the attendant risks of any surgery with potential complications ranging from micronutrient deficiencies, to adhesions and death (Tomycz *et al.*, 2012).

Given the prevalence of obesity there has been an increasing focus on lifestyle-based strategies as a means of preventing and treating excess weight gain. In particular, strategies that are more time efficient and easy to follow. One increasingly popular strategy is the concept of intermittent fasting (IF). Intermittent fasting is any strategy that uses periods of severe or total energy restriction for short periods (typically 18-24 hours) interspersed with periods of normal energy intake. The use of severe vs. total energy restriction is currently preferred as the 2 protocols produce analogous metabolic adaptions to each other, while being more easily tolerated by participants. Of the intermittent fasting strategies currently in use, the most actively studied is the modified alternate day fast (*m*ADF) where participants eat normally one day and then severely restrict energy intake (typically 500-600 kcal per day or ~25% of normal intake) the next in a continuous alternating pattern. The alternate day fast has proven to be as effective, or almost as effective, for weight loss and the improvement of metabolic and cardiovascular risk factors associated with obesity when compared to energy restriction alone (Trepanowski *et al.*, 2017). Another intermittent fasting strategy is the 5:2 diet where practitioners fast for two non-consecutive days of the week (less than 500-600 kcal per day) and eat normally for the remaining 5 days of the week. This form of IF (and 5:2 pattern fasting) has been marketed as an easier to follow protocol that shares many of the benefits associated with *m*ADF and energy restriction diets, however, there have been limited peer reviewed studies comparing the 5:2 diet and other CR diets on weight loss.

In addition to the reduction of energy consumption there is also a need to increase physical activity within the general community (WHO, 2010). Exercise has long been shown to enhance weight loss, however the most commonly reported barrier to exercise participation is a cited lack of time (Del Corral *et al.*, 2009). Current World Health Organisation recommendations are that people undertake between 150 - 300 min of moderate exercise per week for maintenance of health and the reduction of metabolic and cardiovascular risk factors (WHO, 2010). However, the majority of the adult population fails to reach these targets and more time efficient protocols such as SIT and HIIT are gaining popularity as strategies for increasing exercise participation (Sallis *et al.*, 2016). This has led to an increased interest in promoting exercise strategies that require less time commitment, are more varied and yet can stimulate similar metabolic benefits when compared to more traditional training recommendations such as moderate intensity continuous training (MICT). For this reason, strategies such as high intensity (HIIT) and sprint interval training (SIT) have been the subject of a great deal of attention in both the scientific and lay population (Gibala *et al.*, 2014).

Both HIIT and SIT use brief periods of intense exercise, interspersed with rest periods over a number of cycles. HIIT protocols are performed at sub-maximal intensity (typically at 70-90% of VO_{2max}), usually with an exercise interval of 30s or more, where SIT protocols are performed at supra-maximal intensity (>100% VO_{2max}) with exercise intervals of between 8 – 30 s (Keating *et al.*, 2017). In comparison, MICT is usually performed at a steady state over periods of 30 – 60 minutes at an intensity of approximately 40 - 59% of VO_{2max} and/or 55 - 69% of predicted maximal heart rate (HR_{max}) (Norton *et al.*, 2010).

In terms of outcomes both SIT and HIIT demonstrate similar or at times superior effects on aerobic fitness, cardiovascular and metabolic risk factors in comparison to MICT, however exercise alone is rarely able to produce substantial changes to adiposity

(Shaw *et al.*, 2006). This potentially opens the way for these protocols to be used in combination with either IF or CR to promote more significant weight loss and general health benefits, however to date, there have been few studies investigating these interactions. For this reason, the current study will test the hypothesis that the combination of the 5:2 intermittent fasting diet and SIT will have an additive effect in reducing fat mass, body mass and in preventing the loss of lean mass that is often seen with energy restriction strategies. Additionally, this study will test the hypothesis that the 5:2 diet will produce comparable body mass, fat mass and visceral fat mass reductions to more established energy restriction protocols. Thus, this study aims to investigate the effects of SIT and IF on body composition and markers of metabolic and cardiovascular health, both in combination and in isolation to determine if there is any evidence of synergism between the two strategies. Additionally, this study aims to demonstrate the efficacy of the 5:2 diet for body/fat mass reduction in a free-living population.

Chapter 2 – Literature Review

2.1 Introduction

Obesity rates have increased globally for the past few decades and have almost tripled since 1975, with approximately 39% of the world's adult population being recently categorised as overweight and a further 13% described as obese (body mass index (BMI) > 25 kg/m^2) in 2016 (WHO, 2018). While it would be logical to assume that this problem is confined to industrialised nations such as the U.S.A where roughly 1/3 of men and women are classified as obese or overweight, there are many developing nations whose obesity rates are similar (or even higher). For example, the percentage of people classified as overweight and obese was over 50% in Micronesia, Kuwait, Tonga, Samoa and Qatar in 2013 (Ng *et al.*, 2014). While this seems paradoxical, increased globalisation and associated elevated energy intake caused by the ready availability of affordable energy dense foods, in combination with decreasing levels of physical activity, appear to be the main cause. However, changes in gut microbiota and several other factors associated with increased urbanisation (decreased sleep duration, noise/light pollution etc.) have been also been implicated (Malik *et al.*, 2013; Ng *et al.*, 2014). Additionally, there may be a number of epigenetic and psychological factors at play (Gluckman & Hanson, 2004; Zschucke *et al.*, 2013).

The costs associated with treating obesity and its associated co-morbidities (such as Type 2 diabetes and metabolic syndrome) has increased pressure on medical systems worldwide. Thus, reducing the burden of obesity and metabolic disease by addressing the factors underlying these trends has become a matter of urgency to reduce the impact on affected nations.

Current strategies for the reduction of obesity revolve around lifestyle interventions, an approach that focuses on modifying energy balance, specifically, a positive energy

balance resulting from increased energy intake and lower energy output (sedentary lifestyle). This approach involves the prescription of daily energy restriction (typically ~20% reduction of RDI) combined with moderate intensity continuous exercise training to halt or reverse the persistent, long term positive energy balance (WHO, 2010; Okely *et al.*, 2012).

While controlled laboratory studies have demonstrated the efficacy of energy restriction it often suffers from poor compliance over the longer term in free-living populations, particularly in younger individuals (<60 years of age). This is largely due to a combination of negative peer influence and fast-food consumption at social gatherings (Khan *et al.*, 2014). Another common cause of poor compliance in these strategies is the time commitments associated with exercise and the preparation of energy restricted diets (Del Corral *et al.*, 2009). Excluding these external factors there are additional, hormonal determinants of compliance, particularly the hormones leptin and ghrelin. Leptin is the hormone that is responsible for controlling satiety and for opposing the actions of ghrelin (stimulation of hunger). Under normal conditions, when energy stores are depleted, leptin levels decrease and hunger increasing allowing the body to maintain a stable weight. However, in people suffering obesity leptin levels become persistently high as the body becomes resistant to leptin signalling, decreasing inhibition of hunger signals leading to increased food intake due to increased ghrelin signalling as a result (Pan & Meyers, 2018). For these reasons there has been a great deal of focus on alternative diet and exercise strategies that improve compliance.

One such strategy is the use of time efficient high intensity interval training (HIIT) or sprint interval training (SIT) protocols. The decreased time commitment associated with these protocols (<90 min per week) is advantageous when compared with current recommendations for MICT that prescribe 150 minutes of exercise per week (Gillen & Gibala, 2014). High intensity interval training is a strategy using low volume (4-6 intervals), high intensity exercise performed in bursts of approximately 30 sec at near

maximal aerobic intensity (~70-90% VO_{2max}) followed by 4-5 minutes of active rest over a 30-minute period. Sprint interval training is similar to HIIT but is typically performed with smaller interval intervals (8-30s) and at supramaximal intensity (>100% VO_{2max}). Both protocols have been demonstrated to produce similar benefits in terms of glucose tolerance, insulin sensitivity, mitochondrial function and improved VO_{2max} but with a much smaller time commitment (Ma *et al.*, 2013; Gillen *et al.*, 2014).

Another strategy focussed on dietary manipulation that is gaining popularity is the practice of intermittent fasting. These interventions involve periods of normal eating interspersed with periods of intense energy restriction. These protocols reduce the need for tedious calorie counting and are generally well tolerated within participant groups in comparison to continuous energy restriction. These protocols have been demonstrated to be effective for weight loss and the improvement of other metabolic and cardiovascular risk factors associated with obesity (Varady, 2011; Trepanowski *et al.*, 2017). This review will examine intermittent fasting and both HIIT and SIT protocols, alone and in combination and their beneficial effects on these variables.

2.2 Energy Restriction Strategies for Weight Loss

2.2.1 Continuous Energy Restriction

The most commonly prescribed type of dietary intervention for obesity is long-term continuous energy restriction (CER) in combination with regular exercise and other lifestyle changes. Continuous energy restriction refers to most diets that are traditionally used for weight loss and simply uses decreased calorie intake over a period of months or years, with individuals eating an energy restricted diet (60-80% of recommended daily intake for size (RDI)) to alter their energy balance towards negative energy balance, thus eliciting weight loss (Barnosky *et al.*, 2014; Varady & Hellerstein, 2008).

Chronic CR improves blood lipid profiles (human/animal), increases longevity (rodents/drosophila), reduces oxidative stress (human/animal), reduce total body fat percentage (human/animal), reduce blood pressure (human/animal), reduce heart rate (rodents), provides increased neuroprotection and reduces the incidence of some cancers (human/animal) (Mager *et al.*, 2006; Lee & Longo, 2011; Kroeger *et al.*, 2012). Increased lifespan and the cardio-protective effects of energy restriction have been linked to reductions in oxidative damage, reduced levels of insulin and glucose within the blood, increased vagal tone to the heart and protective effects derived from sensitisation to low intensity stress stimuli (hormesis) (Masoro, 2000; Mager *et al.,* 2006). While the neuroprotective qualities of energy restriction have been linked to increased expression of Sirtuin-1 (SIRT1) deacetylase, brain-derived neurotrophic factor (BDNF) and heat shock protein 70 in animal models (Cohen *et al.,* 2004; Qin *et al.,* 2006; Tajes *et al.,* 2010). There is continuing discussion as to the mechanisms behind these beneficial health effects, with several possible processes implicated, particularly in terms of neuroprotection, cardiovascular improvements and anti-aging effects.

Continuous energy restriction, while effective in controlled studies, is often subject to poor compliance in the long term (over 6 months) in unmonitored and controlled environments. This is largely due to the onerous nature of daily calorie counting, societal pressures and self-selection of foods during the dietary period (Sallis *et al.*, 1990; Khan *et al.,* 2014; Emadian *et al*., 2015). These, and hormonal factors, necessitate the search for alternative strategies that provide similar benefits with an easier integration into the modern lifestyle.

2.2.2 Ketogenic Diets

Ketogenic diets are a sub-category of CR diet that specifically restrict carbohydrate (~20-30 g/day) intake to force the body into ketogenesis and promote fat oxidation for

its energy needs (Foster *et al*., 2003; Samaha *et al*., 2003; Katz, 2005; Goldstein *et al*., 2011). In comparison to diets that use CR alone, ketogenic diets produce greater weight reduction in the early stages of the diet (≤ 6 months), however these differences are no longer apparent by 12 months, with much of the initial weight loss attributed to decreased water retention (Katz, 2005; Goldstein *et al*., 2011; Atallah *et al*., 2014). There have also been some reports of poor compliance due to the carbohydrate restriction associated with the ketogenic diets. One study (Goldstein *et al*., 2011) noting that many participants were exceeding the prescribed carbohydrate intake, often by more than twice the recommended value due to cultural factors (subjects were accustomed to a Mediterranean diet) and the restricted food choices available. Goldstein *et al*. (2011) also noted that participants continued to lose weight due to voluntary energy restriction (energy consumption was not limited in this study), noting that this weight loss could also be influenced by the increased energy cost associated with the use of proteins for gluconeogenesis (Paoli *et al*., 2013). There is a need for more studies assessing the safety of low carbohydrate diets, as despite appearing to be relatively safe these diets carry an increased risk of renal lithogenesis, particularly uric acid stones, and have been demonstrated to produce instances of insulin resistance, glucose intolerance and dyslipidaemia in rats (Foster *et al*., 2003; Shulsinger & Penniston, 2015).

2.3 Fasting Strategies

2.3.1 Natural fasting events

Periods of feast and famine have been a common feature of the human experience and our evolution, especially in hunter-gatherer and subsistence cultures where sustenance was determined by the success of farmed crops and the hunting and gathering of sufficient foods in an environment where food availability is subject to

seasonal variation. For this reason, the human metabolism has evolved in such a way that it is well adapted to coping with periods of fasting interspersed with periods of seasonal abundance.

In the last century the world suffered through the Great Depression and the subsequent restrictions to nutrition and dietary energy intake that it caused. Health professionals at the time predicted that the life span of people during those years would decrease rather than increase, as they did, which led to scientific interest in fasting as a means of improving health (Granados & Roux 2009).

Another significant event of the previous century was World War II, where food shortages were a relatively common feature of day to day life. One particular event that has been well studied is the survivors of the Dutch Hunger Winter in the winter of 1944-45, where food supplies were cut-off from reaching the west of the country by the occupying German forces. This led to food rations being restricted largely to potato and bread, with the average daily intake of roughly 1000 kcal/day in November of 1944 and dropping even further to approximately 500 kcal by April of 1945 (Lumey *et al.*, 2007). Following the war it was noted by researchers that there was negative effects on a number of mental and physical health parameters in individuals who had been exposed to the famine *in utero* (particularly in early gestation) with an increases in schizophrenia risk, increased risk of adult obesity, altered blood lipid profiles and cardiovascular disease (Lumey *et al.*, 2007; Schulz, 2010). The Dutch Hunger Winter provided some of the first evidence of maternal predictive adaptive responses (PARs) and has helped to increase our understanding of how the nutritional environment, particularly *in utero* can influence subsequent adult weight gain.

2.3.2 Religious Fasting

Fasting has long been a mainstay of religious practice and is often a preparatory rite for major festivals or other rituals. The most commonly known fasting rituals include the Ramadan fast of Islam, the Lenten fast of Catholicism and the Yom Kippur fast of Judaism. These fasting practices are commonly used either as a remembrance or, in the case of Catholicism, in preparation for the Catholic communion rite (Schmemann, 1959).

2.3.2.1 Ramadan

The Ramadan fast (RIF) is practiced during the ninth month of the Islamic lunar calendar to commemorate the revelation of the Quran to the prophet Mohammed. The Ramadan fast is a total fast (100% energy restriction) that lasts from sunrise to sunset and is obligatory for all Muslims unless they are ill, pregnant, travelling or otherwise at risk due to fasting. The fast is preceded by the morning meal (Sahour) and is ended after sunset with the evening meal (Iftar) with meals traditionally containing higher levels of fat, carbohydrate and animal derived proteins than the meals consumed outside of Ramadan, most likely as a strategy to prolong satiety (Chaouachi *et al.*, 2009). The RIF has been causatively associated with decreased BMI, total cholesterol, triglycerides, plasma glucose and total body fat (Adlouni et al., 1997; Larijani eta al., 2003; Akhtaruzzaman et al., 2014). However, other investigations have found there to be no significant changes in these parameters (Maislos *et al.*, 1998; Ramadan & Barac-Nieto, 2000; Afrasiabi et al., 2003; Roky et al., 2004). The main difference in outcome appears to be the methodologies involved, as eating patterns for RIF differ due to seasonal, regional, and socio- economic variation and with some cultures having their own dietary prescriptions (e.g. Bedouin tribes excluding the morning meal entirely) (Maislos *et al.*, 1998; Karaağaoğlu & Yücecan, 2000; Trepanowski & Bloomer, 2010).

2.3.2.2 Other Religious fasts

Another example of religious intermittent fasting is the Lenten fast practiced by adherents of the Catholic and Orthodox faiths during the six weeks leading up to Easter. Two distinct fasts are undertaken during Lent; the first is a total fast on both Wednesday and Friday combined with a second fast on Saturday and Sunday which is a "fast of abstinence" that ends upon the reception of Holy Communion (Merz, 2014). The prevalence of Lenten fasting is in decline and therefore little is known of its effects on those participating due to the lack of research on the subject.

2.3.3 Fasting Strategies for Weight Loss

2.3.3.1 Alternate Day Fasting

Alternate day fasting (ADF) uses alternating "feast" and "fast" days to achieve its effects. On fast days participants do not consume any food for 20-24 hours but are permitted to consume sugar free gum and nutrient free beverages (black tea and coffee) while maintaining a high intake of water. On feast days participants are permitted to consume their normal food intake or double their normal intake to maintain body weight (Halberg *et al.*, 2005; Heilbronn *et al.*, 2005). Heilbronn *et al.* (2005) noted a 2.5% decreases in body weight and fasting insulin levels during their 22-day study while Halberg *et al.* (2005) noted that body weight remained stable in male participants undertaking 14 days of ADF in addition to improvements to inulin-mediated glucose uptake. Heilbronn *et al.* (2005) reported that hunger on fasting days did not decrease over time and suggested that adding a small meal on fasting days might improve the overall suitability and ease of application of this strategy in populations suffering from obesity over extended periods of time.

2.3.3.2 Modified Alternate Day Fasting

The modified alternate day fast is an intermittent energy restriction (IER) strategy that involves an alternating cycle of restricted energy intake (as little as 25% of recommended daily intake (RDI)) on fasting days, with *ad libitum* food intake on subsequent days (Varady & Hellerstein, 2008; Lee & Longo, 2011; Varady *et al.*, 2015). Interestingly, despite being able to eat *ad libitum* or consume up to 125% of their RDI on "feed" days there appears to be little in the way of compensatory overeating with participants typically consuming between 100-110% of their RDI on these days (Varady, 2011).

Although slightly less effective for weight loss alternate day fasting (*m*ADF) demonstrates similar health benefits when compared to CR (Varady, 2011; Barnosky *et al.*, 2014). Additionally, ADF was thought to be better tolerated in terms of compliance, though a recent study has provided some evidence that *m*ADF is influenced by reduced dietary adherence when compared to long-term daily CR (Trepanowski et al., 2017).

2.3.3.3 The 5:2 Intermittent Fasting Diet

The 5:2 intermittent fasting diet is an IER strategy that is becoming increasingly popular, particularly within the lifestyle, health and fitness industries. The 5:2 diet consists of two days of fasting per week with energy intake limited to 500 Kcal for women and 600 Kcal for men. These days are non-consecutive with *ad libitum* food intake during the remaining five days (Brown *et al.*, 2013). The 5:2 diet has been marketed as being as effective as long-term moderate energy restriction (Mosely & Spencer, 2013) in the treatment of obesity through weight loss. However, there are few randomised control studies to support these proposed benefits, with the beneficial effects currently attributed to the 5:2 diet largely extrapolated from data relating to the *m*ADF diet (Brown *et al.*, 2013; Longo & Panda, 2016). A recent paper by Schübel

et al. (2018) has reported decreases in body weight that were slightly higher, but still comparable to, participants undertaking daily energy restriction as well as improvements to total cholesterol, LDL, HDL, triglycerides, HOMA2-IR, insulin and leptin. However, more randomised control studies are required to confirm these reported benefits, particularly in terms of long-adherence as there is currently only a single study available assessing this, with the 5:2 diet demonstrating a 50% lower dropout rate over 50w than CCR (Schübel *et al.*, 2018).

2.3.3.4 Other 5:2 fasting strategies

While the most widely marketed 5:2 strategy is the 5:2 diet (Brown *et al.*, 2013) there have been other studies that use two consecutive days of fasting rather than the two non-consecutive days seen in the 5:2 diet (Harvie *et al.*, 2011; Harvie *et al.*, 2013; Antoni *et al.*, 2016). These studies investigated the effects of these protocols in overweight women and found that this protocol was equally as effective for weight loss as CER (Harvie *et al.*, 2011) and that combining it with either carbohydrate restriction or *ad libitum* protein & fat consumption led to significantly increased weight loss when compared to CER (Harvie *et al.*, 2013). Additionally, Harvie *et al.* (2013) demonstrated significant reductions in C-reactive protein, LDL, total cholesterol, leptin, insulin resistance, triglycerides & blood pressure.

2.3.3.5 Periodic fasting, fasting mimicking diets and juice fasting

Periodic fasting (PF) refers to a form of IER strategy that uses more extended periods of total or near total fasting (<200 kcal per day or water only) than intermittent fasting strategies. Periodic fasts are also carried out over several consecutive days (typically 7-14) repeated at intervals of greater than 2 weeks (Longo & Mattson, 2014). This type

of fasting has been used clinically to treat rheumatoid arthritis (RA) due to reductions in inflammatory mediators potentiated by the absence of arachidonic acid derived from animal derived food products. Typically, these diets use a total fast (water only) followed by the subsequent adoption of a vegetarian diet (Müller *et al.*, 2001; Sher *et al.*, 2016). This type of fasting is also associated with decreases in glucose levels, CRP, blood pressure, body weight, abdominal fat, insulin, IGF-1 levels and increases in insulin sensitivity and stress resistance (Longo & Mattson, 2014; Brandhorst *et al.*, 2015).

Another type of PF that is popular amongst the general population is juice fasting, where all meals and snacks are replaced with either fruit or vegetable juices, typically performed over several days. Juice fasting results in a considerable energy restriction, amounting to a daily energy intake of <350 kcal per day during either a 7- or 8-day fast (Huber *et al.*, 2003 Michalsen *et al.*, 2005). Studies report that participants experienced significant increases in quality of life with accompanying decreases in pain levels (measured by SF-36 questionnaire or visual analogue scale of pain) while also experiencing beneficial changes to total cholesterol, phospholipids, insulin, CRP, triglycerides, LDL cholesterol and body weight in the general population (Huber *et al.*, 2003 Michalsen *et al.*, 2005). Due to the severity of energy restriction it is unlikely that juice fasting is a viable long-term strategy for weight loss unless followed by subsequent alterations to daily caloric intake (as seen in RA treatment) as the parameters measured would rapidly return to their baseline values (Huber *et al.* (2003).

Due to a number of concerns surrounding water only fasting and the use of PF strategies which allow very low-energy intake over a number of days, a new fasting strategy called the fasting mimicking diet (FMD) was developed to increase both the safety and level of dietary compliance within participants. The FMD is a vegetarian style IER diet with a macronutrient and micronutrient composition designed to produce similar effects to PF while providing sufficient nourishment to minimise the adverse side

effects associated with such an extreme low-energy program. The fast is carried out over 5 days using commercially produced food, packaged specifically for each day of the fast with a caloric allowance of approximately 1090 kcal on the first day and with an approximate intake of 725 kcal on subsequent days. The FMD provides similar benefits to PF (decreased inflammation, plasma glucose, body weight and trunk fat), with participants demonstrating a high level of dietary compliance (Brandhorst *et al.*, 2015; Longo & Mattson, 2014). A recent study (Antoni *et al.*, 2016), demonstrated in a 3-way crossover protocol that partial (75% restriction) rather than total (100% restriction) energy restriction was preferable for producing favourable alterations to lipid and glucose metabolism following a 36 hour fast due to the amelioration of fasting-related reductions in glucose tolerance. It also appears that bouts of severe energy restriction create disturbances in energy substrate utilisation that lead to a greater degree of metabolic flexibility, and consequently, increased metabolic health in comparison to CER (Antoni *et al.*, 2016).

2.3.3.6 Meal Skipping

Meal skipping (particularly breakfast) is reported to be one of the first modifications that people make to their diet when attempting to lose weight, despite it generally being perceived as a negative behaviour for weight loss, both anecdotally and within the scientific literature. This is due to associations between meal skipping and increased BMI, increased fat mass and lipid dysregulation in a number of studies (Geliebter *et al.*, 2014; Leidy *et al.*, 2015). There are several proposed mechanisms for these associations including perturbations of the circadian rhythm, decreased dietary β-glucan content, reduced satiety, poorer nutritional choices, inadequate micronutrient intake and increased impulsive snacking. However, to date, there have been insufficient controlled studies to confirm or refute causation in each case (Levitsky & Pacanowski 2013, Geliebter *et al.*, 2014; Mattson *et al.*, 2014; Leidy *et al.*, 2015).

The link between increased BMI and meal skipping has been suggested to be due to breakfast eaters being less susceptible to impulsive snacking as a result of increased satiety, which in turn would limit the poorer nutritional choices that often arise with impulsive snacking (Schlundt *et al.*, 1992; Dhurandhar *et al.*, 2014). Alternatively, it has also been suggested that timing of energy consumption might be a contributing factor due to possible metabolic differences in the consumption of several small meals over the day, as compared to a smaller number of large meals where there is little difference in total energy intake (Schlundt *et al.*, 1992). More randomised control studies are needed to elucidate the mechanisms and to separate causation from correlation before the value of recommending breakfast consumption or breakfast skipping as weight loss strategies.

2.3.3.7 Starvation and moderate duration acaloric fasting

Despite the negative association's society places on starvation, our metabolism is well adapted to deal with short to moderate periods of total food deprivation with little in the way of ill effects. Given that the physiological adaptions to food deprivation in starvation and moderate term acaloric fasting are essentially the same they will be addressed together. During the first 7 days of food deprivation there is usually a period of accelerated weight loss associated with alterations to the body's sodium balance (Kerndt *et al.*, 1982). During this time liver glycogen is depleted and the body predominantly utilises gluconeogenesis to support its energy requirements through the production of glucose from the oxidation of glycerol, amino acid and protein sources (Kerndt *et al.*, 1982; Consoli *et al.*, 1987).

The use of gluconeogenesis for glucose production has the effect of producing ketone bodies as a by-product, which as food deprivation continues become the preferred energy substrate used by the CNS to maintain function, as well as providing a measure

of protection against neural damage associated with the increases in oxidative stress reported in both humans and rodents during prolonged fasting (Mattson *et al.*, 2002).

As the body transitions from glucose metabolism insulin levels reduce and the body decreases its rates of gluconeogenesis, resulting in a decrease in the breakdown of proteins for energy production, sparing its remaining protein reserves to preserve other physiological functions (Kerndt *et al.*, 1982).

Moderate term acaloric fasting and starvation have not been recommended as medical interventions for many years due to concerns regarding safety vs. efficacy given the wide range of negative side effects associated with them such as: light-headedness, protein loss, abdominal pain, oedema, weakness, orthostatic hypotension, metabolic acidosis and death (Kerndt *et al.*, 1982). Death from acaloric fasting is rare due to its prolonged nature but can occur due to severe ventricular arrhythmias and renal failure caused by underlying conditions or dietary factors (Kerndt *et al.*, 1982). While acaloric fasting is clearly not a long-term solution it could be useful for “resetting” metabolism and providing acute positive changes to metabolic and cardiovascular risk factors.

2.4 Physiological and metabolic benefits of Intermittent Fasting

Irrespective of the specific regimes, IF has consistently demonstrated improvement in a number of health related parameters including glucose metabolism (human/rodent), insulin sensitivity (human/rodent), decreased insulin secretion (human/rodent), reduced inflammatory markers (human/rodent), decreased insulin-like growth factor (IGF-1) secretion (human/rodent), improved blood lipid profiles (human/rodent), increased fat oxidation (human/rodent), increased longevity (animal/rodent), increased alertness (animal/rodent), increased ischaemic protection (rodent), increased neurogenesis (animal/rodent) and reduced oxidative stress (animal/rodent) (Mattson *et al.*, 2002; Mitchell *et al.*, 2010; Varady *et al.*, 2013; Jongbloed *et al.*, 2014; Brandhorst *et al.*,

2015). Additionally, there have been reports that IF could potentially decrease the occurrence of seizures in epilepsy patients after being partially successful in mice (Hartman *et al.*, 2010). A summary of the beneficial effects reported for IF can be found in Table 2.1.

Table 2.1. - Summary table of beneficial effects attributed to Intermittent Fasting.

Author	Benefit	Species	Proposed mechanism/Improvements in study parameters
Ahmet et al., 2005	Cardio-protection following ischaemia-reperfusion injury	R	↓ M.I. size, ↓ Neutrophil and Macrophage infiltration, ↓ Ventricular remodelling, ↑ Survival
Anson et al., 2003	Resistance to excitotoxic neuronal injury	M	↓ Glucose, ↓ Insulin, ↑ IGF-1, ↑ β-hydroxybutyrate, ↑ Neuronal survival
Arumugam et al., 2010	Neuroprotection following induced ischaemic brain injury	M	↓ Neurological impairment, ↓ Infarct size, ↑ Neurotrophic Factors, ↑ Stress response proteins, ↓ Proinflammatory Cytokines, ↑ Survival
Ash et al., 2003	Improved Glycaemic control in individuals with type 2 diabetes	H	↓ Body weight, ↓ Fat mass, ↓ HBA1c, ↓ TG
Baumeier et al., 2015	Variation in Hepatic lipid droplet composition leading to Diabetes prevention	M	↓ Fat mass, ↓ Insulin, ↑ Insulin Sensitivity, ↑ Metabolic Flexibility, ↑ Fatty Acid Oxidation, ↓ Hepatic diacylglycerol,
Campos-Rodriguez et al., 2016	Improved outcomes to GIT infection with *Salmonella typhimurium*	M	↓ Intestinal/Systemic Bacterial Load, ↑ IgA, ↓ Bacterial Colonisation,
Carlson and Hoelzel, 1946	Longevity	R	↑ Lifespan with fasting, ↓ Body weight, ↓ Mammary tumour formation
Carter et al., 2016	Improved Glycaemic control in individuals with type 2 diabetes	H	↓ Body weight, ↓ HBA1c
Eshghinia & Mohammadzadeh, 2013	Improvements to biomarkers associated with CVD & Metabolic disease	H	↓ Body weight, ↓ Fat mass, ↓ BP, ↓ BMI, ↓ Waist circumference
Godar et al., 2015	Cardio-protection following induced ischaemic injury	M	↑ Autophagy, ↓ Infarct size, ↓ Cardiac remodelling
Gotthardt et al., 2016	Improved Metabolic Health	M	↑ Glucose tolerance, ↑ Insulin tolerance, ↓ Body weight, ↓ Fat mass, ↓ Insulin, ↓ Leptin
Halagappa et al., 2007	Mitigation of age-related decline in cognitive functioning in a model of AD	M	↑ Open field activity, ↑ Performance in Morris Water task
Halberg et al., 2005	Increased insulin mediated glucose uptake	H	↑ Insulin Sensitivity, ↑ Adiponectin, ↑ Inhibition of lipolysis by insulin
Harvie et al., 2011	Improvements to biomarkers associated with CVD, metabolic disease and cancer	H	↓ Body weight, ↓ Insulin resistance, ↓Inflammatory markers, ↓ leptin, ↓ blood lipids, ↓ fasting insulin, ↑ ghrelin
Harvie et al., 2013	Improvements to biomarkers associated with CVD & Metabolic disease	H	↓ Body weight, ↓ insulin/insulin resistance, ↓ leptin, ↓ IL-6, ↓Total Cholesterol/TG, ↓ SBP/DBP, ↓ Body fat
Honjoh et al., 2009	Increased Longevity	RW	RHEB-1 & TOR signalling involved in the induction of genes controlling fasting induced longevity. ↑ Oxidative/heat stress resistance,
Karbowska & Kochan, 2012	IF mediated upregulation of gene expression associated with efficient fat storage	R	↓ Body weight, ↓ Epididymal Fat, ↓ Leptin, ↓ TG, ↑*Fsp27,* ↑ MCPT1, ↑ PPARϒ2, ↑ C/EBPα
Katare et al., 2009	Cardio-protection following ischaemia-reperfusion injury	R	↓ Myocardial remodelling, ↑ Preservation of cardiac volumes/Function, ↑ Expression of angiogenic factors, ↑ Arteriole/Capillary density, ↑ Survival
Kim et al., 2017	Increased adipose thermogenesis and improved metabolic biomarkers	M	↑ Browning of white adipose tissue
Klempel *et al.*, 2012	Reduced risk of coronary heart disease/Weight loss	H	↓ LDL, ↓ TG, ↓ proportion of small LDL, ↓ VAT
Kroeger *et al.*, 2012	Reduced risk of coronary heart disease/Weight loss	H	↓ proatherogenic adipokines, ↓ Plasma lipids, ↑ LDL Particle size
Kumar et al., 2009	Neuroprotection following induced excitotoxic brain injury	R	↑ PSA-NCAM,↑ BDNF, ↑ NT-3, ↑ neuronal survival, ↑ Neurogenesis
Lee et al., 2006	Hippocampal neuroprotection from excitotoxic challenge	R	↑ IFN-γ, ↑ IFN-γ receptor, ↑ Neuronal survival after challenge with glutamate,
Li et al., 2013	Improved Brain function and structures	M	↓ Oxidative stress, ↑ Context based freezing behaviour, ↑ Performance in Barnes maze test, ↑ CA1 pyramidal cell layer, ↑ Drebrin/Synaptophysin
Mager *et al*, 2006	Cardiovascular	R	↑ Vagal tone ↓ Sympathetic activity
Manzanero et al., 2014	Neuroprotection and attenuation of neurogenesis following induced ischaemic brain injury	M	↓ Leptin, ↓ Infarct size, ↑ Hippocampal/subventricular zone basal cell proliferation, ↓ Neuronal cell death
Mardorsky et al., 2009	Neuroprotection in neuropathic mouse model	M	↑ Motor function, ↑ Grip strength, Improved nerve structure, ↑ Myelin protein expression, ↑ HSP70,
Mitchell et al., 2010	increased renal Hepatic function following induced ischaemia-reperfusion injury	M	↑ Acute stress resistance, ↓ Insulin/IGF-1 signalling, ↑ Insulin sensitivity, ↑ Antioxidant defence, ↑ Survival
Rocha et al., 2002	Anti-promoting effect on induced hepatic carcinogenesis in rats	R	↓ Gross liver changes, ↓ Liver nodules, ↓ Size of nodules, ↓ Preneoplastic foci
Tajes *et al.*, 2010	Neuroprotection	M	↑ SIRT1 production
Tikoo et al., 2007	Nephroprotective effect in induced diabetic model	R	↓ Body weight, ↓ BUN, ↓ Increases is BP/Creatinine, ↓ Albumin Depletion, ↓ Lipid peroxidation, ↑ SOD, ↓ Glomerular Damage, ↓ *p53*, ↑ *Sir2*
Trepanowski et al., 2017	Improvements to biomarkers associated with CVD & Metabolic disease	H	↓ Body weight, ↑ HDL, ↓ SBP/DBP, ↓ Body fat, ↓ fasting insulin/insulin resistance, ↓TG, ↓ VAT
Varady et al., 2007	Reduced inguinal and epididymal adipocyte size	M	↑ TG metabolism, ↑ Lipolysis in inguinal fat depot, ↑ *De novo* lipogenesis, ↑ glyceroneogenesis, ↑ FFA
Varady et al., 2011	Improvements to biomarkers associated with CHD.	H	↓ Body weight, ↓ LDL/TG, ↑ LDL Particle size
Varady et al., 2013	Improvements to biomarkers associated with CVD & Metabolic disease	H	↓ Body weight, ↓ TG, ↑LDL Particle size, ↓ Fat mass, ↓ CRP, ↓ Leptin, ↑ Adiponectin
Varady et al., 2015	Improvements to biomarkers associated with CVD & Metabolic disease	H	↓ Body weight, ↓ TG/LDL/TC, ↓ Fat mass, ↓ FFA, ↓ BMI, ↓ Waist circumference
Vasconcelos et al., 2014	Neuroprotection following induction of systemic inflammatory challenge (LPS)	R	↓ Performance in Barnes maze test, ↑ Cognitive performance, ↑ Translocation of NF-κB, ↓ Proinflammatory cytokines, Maintained BDNF levels
Wan et al., 2003	Improvements to biomarkers associated with CVD & Metabolic disease	R	↓ Body weight, ↓ SBP/DBP, ↓ HR, ↓ Body Temperature, ↓ Insulin, ↓ Glucose, ↑ ACTH/Corticosterone
Wan et al., 2010	Cardio-protection following ischaemia-reperfusion injury	R	↑ Adiponectin, ↓M.I. size, ↓ L.V. Wall thickness, ↓ Body weight, ↓ Insulin, ↓ Glucose, ↓ Neutrophil and Macrophage infiltration, ↓ Apoptosis
Williams et al., 1998	Improved Glycaemic control in individuals with type 2 diabetes	H	↓ Body weight, ↓ Fasting glucose, ↓ HBA1c, ↓Total Cholesterol, ↓Triglycerides

Abbreviations: R, Rat; H, Human; M, Mouse; IF, intermittent Fasting; CR, Calorie Restriction; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; SIRT1, Sirtuin1; TG, Triglycerides; VAT, Visceral Adipose Tissue; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FFA, Free Fatty Acids; IGF-1, Insulin-like Growth Factor-1; CRP, C-Reactive Protein; TC, Total, Cholesterol; M.I., Myocardial Infarction; L.V., Left Ventricular; HR, Heart Rate; BMI, Body Mass Index; BDNF, Brain Derived Neurotrophic Factor; NT-3, Neurotrophin-3; PSA-NCAM, Polysialic Acid Neural Cell Adhesion Molecule; TOR, Target of Rapamycin; RHEB-1, Ras homolog enriched in brain-1; SOD, Superoxide Dismutase; BUN, Blood Urea Nitrogen; BP, Blood pressure; MCPT1, M isoform of carnitine palmitoyltransferase 1; PPARγ2, Peroxisome Proliferator-Activated Receptor γ; C/EBPα, CCAAT/enhancer binding protein α; HSP70, Heat Shock Protein 70; IFN-γ, Interferon γ; GIT, Gastrointestinal Tract.

2.4.1 Intermittent fasting and changes to body composition

Intermittent fasting and CR have typically demonstrated equivalent effects on weight loss and metabolism (typically 5-7% of body weight over three months) across different populations. (Varady & Hellerstein, 2007; Barnosky *et al.*, 2014; Harvie & Howell, 2017; Cioffi *et al.,* 2018; Harris *et al.,* 2018). There are however a handful of studies that have noted some variation. A recent, study by Trepanowski and colleagues (2017) in a large cohort reported that *m*ADF produced comparable though slightly less weight loss in comparison to CR in obese adults, over a 12-month period in both sexes. Another large study reported weight loss in obese, premenopausal women (30-45 years) during a 6-month trial using either CR or a weekly fast carried out over two consecutive and 5 days of normal eating (Harvie *et al.,* 2011). In comparison, Schübel *et al.* (2018) demonstrated slightly better weight loss with the 5:2 diet at both 12 and 50 weeks when compared to CR in a large, mixed cohort (*n*= 150; 35-65 years). Greater retention in the IF group vs. CR was reported, with the 5:2 diet being a less stringent fasting regime (less prolonged fasting time) than those used by Harvie *et al.* (2 consecutive days fasting per week) and Trepanowski *et al.* (*m*ADF) with both studies reporting higher dropout rates in their fasting groups vs. calorie restricted groups. Interestingly, a later study by Harvie *et al.* (2013) with a similar cohort size to Trepanowski *et al.* (2017) and a similar fasting protocol to Harvie *et al.* (2011), found that IF had a superior effect in the reduction of body fat when compared to CR while also experiencing a smaller dropout rate over a 4-month period in pre- and post-menopausal women, likely due to differences sex-based and hormonal differences between the cohorts as well as differences in dietary prescription between the studies.

Several other studies and meta-analyses directly comparing IF and CR have found equivalent weight loss between the interventions (Varady *et al.,* 2011; Carter *et al.,* 2016; Trepanowski *et al.,* 2017; Cioffi *et al.,* 2018; Harris *et al.,* 2018). These studies had similar or slightly lower rates of attrition in the IF group, though it should be noted

that these were typically shorter studies (~12 weeks) (Varady *et al.,* 2011; Carter *et al.,* 2016). Taken together these data to indicate that the more stringent fasting regimes might be subject to higher dropout rates in the long term and that the 5:2 diet might be a better choice for success with long term interventions, however there are limited studies making direct comparisons of dropout rates to confirm this.

All studies that reported decreases in body mass also reported concomitant changes to other body composition values including; changes to visceral fat mass, total fat mass and hip/waist/bust measurements (Klempel *et al.*, 2012; Eshghinia & Mohammadzadeh, 2013; Varady *et al.*, 2015; Carter *et al.*, 2016; Schübel *et al.*, 2018). It should be noted, however, that it is common to find uncontrolled human studies in the field of fasting which, in combination with the relatively small number of studies, complicates comparisons between studies. The following studies included above did not include a dedicated control group: Ash *et al.*, 2003; Harvie *et al.*, 2011; Klempel *et al.*, 2012; Eshghinia & Mohammadzadeh, 2013; Harvie *et al.*, 2013; Varady *et al.*, 2015; Carter *et al.*, 2016.

2.4.2 Intermittent fasting and cardiovascular health

Intermittent fasting has been associated with improving cardiovascular health, predominantly through reductions in circulating triglycerides, circulating low density lipoprotein (LDL), decreases in total cholesterol and reductions in pro-atherogenic adipokines (Williams *et al.*, 1998; Kroeger *et al.*, 2012; Varady *et al.*, 2015). Additionally, IF has been shown to reduce adipocyte size which is important due to the association between increased adipocyte size and dysregulation of normal TG storage in mice using a total fast on fasting days (Varady *et al.*, 2007). This dysregulation results in a reduced capacity for the uptake of circulating triglycerides that, in turn, leads to ectopic fat storage and downstream dysregulation of insulin activity and

lipolysis (Larson-Meyer *et al.*, 2006; Varady *et al.*, 2007). Additionally, this ectopic fat storage is accompanied by an increase in inflammatory responses due to enhanced macrophage infiltration of tissues (Larson-Meyer *et al.*, 2006; Varady *et al.*, 2007).

Reductions in circulating lipids in both male and female cohorts are reported following IF/*m*ADF (Williams *et al.*, 1998; Kroeger *et al.*, 2012; Varady *et al.*, 2015; Schübel *et al.*, 2018). Ash *et al.* (2003) were able to show decreases in triglyceride levels but no change in total cholesterol, LDL or HDL levels in males with diabetes (<70 years), using a protocol where participants consumed 1000 Kcal per day (less than half the daily recommendation) on 4 consecutive days of the week, with *ad libitum* food intake on the remaining days. Additionally, Kroeger *et al.* (2012) were able to demonstrate in their uncontrolled study that decreases in circulating lipids appear to be tied to decreases in visceral fat and reductions in both TNF-α and leptin production, likely through increased oxidation of free fatty acids using a single day of fasting during the week. There is also evidence that ADF can reduce the size of inguinal and epididymal adipocytes in mice and to beneficially alter triglyceride (TG) kinetics (Varady *et al.*, 2007).

Another beneficial effect associated with IF is increased levels of high-density lipoprotein (HDL), which in combination with decreased levels of LDL is considered cardio-protective (Varady *et al.*, 2011; Kroeger *et al.*, 2012). High density lipoproteins are reported to be effective in scavenging LDL deposits from tissues and in preventing the formation of atherosclerotic deposits (Badimon *et al.*, 1990). Badimon *et al.* (1990) were able to demonstrate that HDL and very high-density lipoprotein (VHDL) were able to stimulate regression of atherosclerotic lesions in rabbits following 30 days of VDHL & HDL supplementation after consuming a high cholesterol diet for between 30-60 days.

While improvements in HDL levels are commonly reported with IF there are several studies that have reported no significant change in HDL levels. Harvie *et al.* (2011) in

their 6-month uncontrolled study examined the effects of a fasting (2 consecutive days of fasting per week in 107 overweight and obese women (30-45 years)) on markers of metabolic & cardiovascular health. They found that there was no change in HDL levels by the end of 6 months with levels ranging from 1.4-1.5 mmol/L at the beginning of the study compared to 1.4-1.6 mmol/L at the end of the study. Similarly, Kroeger *et al.* (2012) in their study examining the effects of a single day of fasting per week using either a food or liquid based dietary approach in 54 overweight and obese women found no change to HDL levels from baseline over an 8-week weight loss period. Another study that examined the effects of fasting on 51 males with diabetes using 4 consecutive days per week of fasting (Ash *et al.,* 2003) also noted that there was no change to HDL levels. While it is difficult to draw any firm conclusions from these studies due to differences in the protocols used in these studies compared to the more comprehensively studied *m*ADF protocol and due to the absence of control groups in several studies (Ash *et al.,* 2003; Harvie *et al.*, 2011; Kroeger *et al.*, 2012; Harvie *et al.*, 2013; Varady *et al.*, 2013).

In addition to these benefits ADF has also been shown to decrease both heart rate (HR) and blood pressure (BP) in rats, two key risk factors in CVD (Mager *et al.*, 2006). While this effect has yet to be demonstrated consistently in human subjects the phenomenon has been reported in obese human subjects maintained on a short-term CR diet (Caviezel *et al.*, 1986). Also, a handful of uncontrolled studies have reported decreases in blood pressure using both *m*ADF and a two day fast (Harvie *et al.*, 2011; Harvie *et al.*, 2013; Varady *et al.*, 2013). Additionally, others like Trepanowski *et al.* (2017) have reported no change to blood pressure values. It is also worth noting that Mager *et al.* (2006) reported that the rat's HR and BP returned to their previous values rapidly once the dietary intervention was withdrawn, which may necessitate long term adherence to IF to maintain these benefits.

Despite many of the published articles reporting positive effects on cardiovascular risk factors there have been a handful of investigations that have raised concerns about the long-term viability of IF. One, Soeters *et al.* (2009), found no difference in blood lipid concentrations, serum glucose or protein metabolism with ADF with100% energy restriction on fast days. This study has several factors that potentially influenced the results, in that the study was uncontrolled, they used relatively lean subjects (BMI 20-25) and recommended that participants consume 130-140% of their RDI on feed days to maintain body weight. The excessive energy consumption imposed on participants combined with the total fast used may be a possible source of variation as the protocols often allow *ad libitum* food consumption on "feed" days, and, while it might be expected that participants might gorge on "feed" days to compensate for the energy lost on fast days it appears that participants choose to consume 100-110% of their RDI on non-fasting days (Varady, 2011). Additionally, this study had both a relatively small male cohort (*n*=8) and a short intervention period of 2 weeks which may also partially account for the results produced.

2.4.3 Intermittent fasting and improvements to metabolic health

One of the most common symptoms associated with obesity and metabolic syndrome is insulin resistance (Black *et al.*, 2013). Insulin stimulation allows cellular uptake of glucose in skeletal muscle and adipose tissue through increased translocation of glucose transporter 4 (GLUT4) to the cell membrane. When glucose uptake is impaired, either through decreased expression of GLUT proteins or through impaired membrane translocation, the body attempts to compensate by increasing insulin secretion (hyperinsulinaemia) to avoid hyperglycaemia. This hypersecretion of insulin can also lead to increased oxidative stress, glucotoxicity and overstimulation of the

pancreatic islets, which in turn, can result in beta cell destruction if hyperglycaemia is sustained over a long period of time (Sakuraba *et al.*, 2002).

In addition to insulin secretion, obesity can influence the insulin receptor complex and its responses. An association between elevated adipokine levels (particularly TNF-α, IL-6 and resistan) in obese patients and the induction of insulin insensitivity has been reported (Park *et al.*, 2005). These adipokines appear to play a role in disrupting signals involved in the translocation of GLUT4 to the cellular membrane (Park *et al.*, 2005). It's unclear whether these adipokines play a causal role in the down regulation of GLUT4 expression in adipocytes as the mechanisms underlying insulin insensitivity are still poorly understood.

Continuous energy restriction increases the expression of GLUT4 in adipocytes, promoting reduced plasma glucose levels and increased insulin sensitivity (Park *et al.*, 2005). The effect of CR appears to be specific to GLUT4 as the expression of other GLUT transporters is unchanged during periods of reduced energy intake (Park *et al.*, 2005; Weiss *et al.*, 2006). ADF/*m*ADF, however, does not influence the extent of GLUT4 translocation. Two studies have examined the effects of ADF on GLUT4 translocation in skeletal muscle with Soeters *et al.* (2009) specifying an energy intake of 130-140% of RDI on non-fasting days to maintain an isocaloric weekly energy intake while Halberg *et al.* (2005) specified that participants eat sufficient food to maintain body weight during the study which may have counteracted some of the effects of fasting. While both studies found no significant difference to controls Halberg *et al.* (2005) were able to demonstrate that IF, like CR, was able to produce improvements in whole body insulin sensitivity, despite the absence of increased GLUT4 expression in skeletal muscle.

Intermittent fasting has consistently demonstrated improved glucose metabolism, decreased circulating insulin, increased glucose tolerance and decreased insulin

resistance using a number of different fasting protocols (Ash *et al.*, 2003; Johnson *et al.*, 2007; Harvie *et al.*, 2011; Klempel *et al.*, 2012; Harvie *et al.*, 2013; Carter *et al.*, 2016; Trepanowski *et al.*, 2017; Schübel *et al.*, 2018). In most cases where these studies included a CR group the results were not significantly different between the CR and IF groups (Ash *et al.*, 2003; Carter *et al.*, 2016; Trepanowski *et al.*, 2017; Schübel *et al.*, 2018). However, Harvie *et al*. (2011 & 2013) in two large studies found slightly greater decreases in HOMA2-IR and fasting insulin levels within their IF cohorts.

There is also some variability within the literature surrounding the effects of IF on adipokines, with many studies reporting decreased levels and a small number reporting no change. Kroeger *et al.* (2012) reported decreases in adiponectin, leptin, IL-6, TNF-α and IGF-1 using a liquid-based 1-day per week IF protocol, with no change in these parameters in the group consuming a food-based IF diet, with the exception of leptin, which decreased in both groups. Harvie *et al.* (2011 & 2013) reported reductions in leptin levels in response to two consecutive days of fasting per week, while Varady *et al.* (2013) reported decreases in leptin and increased adiponectin levels in response to *m*ADF. Johnson *et al.* (2007) demonstrated decreases in serum leptin on energy restricted days but with no significant changes to ghrelin levels on either the energy restricted days or the *ad libitum* eating days. Additionally, Johnson *et al.* reported that TNF-α levels decreased in energy restricted group but not in the fasting group of their study, though these data should be treated with caution as the study was uncontrolled. Schübel *et al.* (2018) reported reductions in adiponectin and leptin levels, but no significant changes to IGF-1, IL-6, TNF-α or other inflammatory markers using the 5:2 diet.

Reduced meal frequency can also influence glucose metabolism in healthy, normal weight individuals. Carlson *et al.* (2007) demonstrated that healthy men and women between the ages of 40 and 55 years who consumed their RDI in one meal per day versus three meals per day experienced elevated fasting glucose levels and impaired

glucose tolerance due to a delayed insulin response. It appears that the maintenance of an isocaloric diet in combination with fasting might influence these factors when compared to the eating patterns that standard *m*ADF and 5:2 protocols usually recommend, in that being isocaloric in nature rather than creating an energy deficit could account for the differences observed in these studies those examining more typical fasting diets. Additionally, the differences in pre-intervention anthropometric profiles (e.g. weight, body fat, BMI etc.) could also result in variable outcomes in response to different interventions.

2.4.4 Effects of intermittent fasting on oxidative stress

A frequently reported benefit of IF and CR is an observed reduction in oxidative stress that has been posited as the possible mechanism underlying some of IF and CR's beneficial effects. Oxidative stress, caused through either the over-production of reactive oxygen species (ROS) or through an inadequate antioxidant defence, can impact tissue function. Oxidative stress is associated with a number of pathological and physiological changes within the body in response to dietary, physical and environmental challenges such as protein damage, neurodegeneration, inflammation, energy dysregulation, insulin resistance, skeletal muscle glucose regulation and skeletal muscle repair (Mattson *et al.*, 2002; Reverter-Branchat *et al.*, 2004; Pinheiro *et al.*, 2010; Andrianjafiniony *et al.*, 2010).

The role of hypocaloric diets in reducing oxidative stress has also been associated with increased longevity in several species including yeasts, roundworms and rodents as many of the detrimental effects of aging appear to be linked to the accumulation of oxidative damage to tissues and cellular repair mechanisms (Hensley *et al.*, 1996; Reverter-Branchat *et al.*, 2004; Honjoh *et al.*, 2009). An example of this is in conditions such as Alzheimer's and Parkinson's disease where symptoms are exacerbated by

damage to lipids, nucleic acid and proteins by reactive oxygen species (Markesbery *et al.*, 2001).

During periods of energy deprivation, it is thought that the observed reductions in oxidative stress are initiated by a mild metabolic stress response, triggered by ER that leads to increased production of protein chaperones and neurotrophic factors that enhances the body's antioxidant defence (Reverter-Branchat *et al.*, 2004; Mattson *et al.*, 2002). Chausse et al. (2015) were able to demonstrate that IF results in tissue specific changes to oxidative balance in the heart, brain, liver and skeletal muscle of 8-week-old Sprague-Dawley rats maintained on a one-month cycle of alternate day fasting. The authors also noted that the liver's respiratory capacity increased in the absence of any change to antioxidant defence or oxidative damage to lipids and nucleic acids. In comparison the authors observed that, in the heart, IF was able to improve redox balance through the prevention of glutathione oxidation, malondialdehyde formation and protein carbonylation (Chausse *et al.*, 2015). Additionally, Chausse *et al.* (2015) reported that ADF was able to downregulate glutathione peroxidase activity without apparent alterations to glutathione redox state or markers of oxidative damage in the heart.

Conversely, Chausse *et al*. (2015) noted that redox balance in the brain and liver was negatively affected by ADF, with increased protein carbonylation (a marker of oxidative stress) without any concomitant increase in mitochondrial capacity. The authors noted that these results were unexpected given that IF is associated with improved brain aging and the partial restoration of antioxidant enzymes lost due to age. The authors suggested that these results were likely due to the young age of the animals used in the study (Chausse *et al*., 2015).

While there have been relatively few studies examining the effects of IF on oxidative stress in human subjects, there have been reductions in secondary oxidative stress

markers such as 8-isoprostanes, nitrotyrosine, protein carbonyls, 4-hyrpxynoneal adducts and advanced oxidation protein products in both small and large studies (Johnson *et al.*, 2007; Harvie *et al.*, 2011; Harvie *et al.*, 2013). However, it should be noted that these studies did not include a control group.

2.4.5 Intermittent fasting and its effects on neurological parameters

The neuro-protective effects of fasting and CR are well documented in animal models and is one of the most exciting areas of research surrounding IF. Currently, these neuro-protective effects are thought to be caused by decreases in oxidative stress and in the up-regulation of neurotrophic factors and sirtuin secretion (Qin *et al.*, 2006). Sirtuins are a family of protein de-acetylases that use nicotinamide adenine dinucleotide (NAD^+) as a substrate to de-acetylate a wide range of acetylated proteins and are involved in metabolic control, age related changes and control of gene expression (Qin *et al.*, 2006).

Intermittent fasting and CER increase circulating levels of sirtuins, particularly SIRT1, which has beneficial effects including increased resistance to physiological stressors, resistance to apoptotic cell death and increased DNA repair (Cohen *et al.*, 2004; Qin *et al.*, 2006). Cohen *et al.* (2004) suggested that SIRT1 could prevent the death of neuronal cells by de-acetylation of Ku70, a DNA repair factor that is closely associated with the pro-apoptotic Bax protein. During incidents of stress or cellular damage acetylation of Ku70 causes it to cease interacting with the Bax protein. This then causes the Bax protein to localise to the mitochondria where it acts as an agonists for the Bcl-2 protein which is responsible for triggering apoptotic cell death (Cohen *et al.*, 2004). Qin *et al.* (2006) found that SIRT1 was able to reduce the occurrence of Alzheimer Disease (AD)-type amyloid neuropathology in mice though down regulation

of Rho kinase (ROCK1) activity, giving IF the potential of being an effective tool in minimising the progression of AD or in the prevention of AD related pathologies.

There is also speculation that IF and CR may have beneficial effects on Parkinson's disease (PD), a condition where dopaminergic neurons in the substantia nigra of the midbrain and neurons in the brain stem degenerate over time (Mattson, 2014). Parkinson's disease appears to be caused by a combination of accumulated oxidative damage and genetic factors. Additionally, there is a demonstrated correlation between metabolic syndrome and increased risk of both AD and PD, predominantly due to increased insulin and IGF-1 resistance (Mattson, 2014). Intermittent fasting has been shown to protect neurons from some of the oxidative and excitotoxic damage that is believed to play a role in the pathogenesis of PD through up-regulating cellular repair and antioxidant defence.

IF has been shown to provide neuroprotection through a number of other mechanisms including increased expression of superoxide dismutase, heat shock proteins, neurotrophic factors and ketogenesis (Duan *et al.*, 2001; Mattson *et al.*, 2003; Davis *et al.,* 2008; Arumugam *et al.*, 2010). Interestingly, despite being toxic when present in high levels (e.g. diabetic ketoacidosis), ketone bodies provide neuroprotection against oxidative damage by acting as a carbon source for the citric acid cycle in the form of acetyl-CoA or succinyl-CoA and through stabilisation of hypoxia-inducible factor-α (Laffel, 1999; Xu *et al.*, 2012). Intermittent fasting has also been shown to increase the secretion of neurotrophic factors such as brain derived neurotrophic factor, nerve growth factor and basic fibroblast growth factor (Mattson, 2014). These neurotrophic factors have all been demonstrated to be protective against a range of metabolic challenges including excitotoxicity, oxidative stress and ischaemic injury (Guo & Mattson, 2000; Kumar *et al.*, 2009). While not fully understood it appears that these neurotrophic factors elicit their protective effect through the upregulation of gene expression related to the production of antioxidant compounds (Guo & Mattson, 2000).

Supervised, prolonged fasting (7 days) has also been demonstrated to improve alertness, provide mood enhancement and analgesia in patients suffering from chronic pain and depression in a long term (13 year) study conducted by Michalsen *et al.* (2006). Separately, Kumar *et al.* (2009) noted increased neural plasticity in the brains of fasted rats following traumatic brain injury while examining the neuroprotective nature of IF. This effect appears to be caused by increased levels of neurotrophic factors stimulating the proliferation of neural progenitor cells and amelioration of oxidative damage. Halagappa *et al.* (2007) also noted that rats maintained on both CR and IF demonstrated greater exploratory behaviour and suggested that such diets might assist in preventing age-related neurological decline. Taken together these studies suggest that some form of IF or CR diet could be an effective treatment or prophylaxis for several neuro-degenerative conditions and may be able to play a role in the prevention or delayed onset of senility.

2.4.6 Effect of intermittent fasting on longevity

Aging is a process whereby an organism progressively loses its ability to respond to physical, mental and environmental stressors and appears to be caused, largely, by the cumulative effects of oxidative damage to cellular components (Koubova & Guarente, 2003; Vasilaki and Jackson 2013). As with the neuro-protective benefits of IF and CR, the effects on longevity have largely been documented in animal studies and are predominantly mediated through chaperone proteins associated with stress responses such as heat shock protein 60 (Hsp60) and Hsp70, increased antioxidant defence and sirtuin mediated down regulation of apoptotic cell death (Reverter-Branchat *et al.*, 2004).

Another process that appears to influence both senescence and life span extension is insulin/IGF-1 signalling. Honjoh *et al.* (2009) were able to demonstrate the importance

of the GTPase RHEB-1 and TOR signalling in the genetic induction of many IF processes. Their study demonstrated that RHEB-1 manipulates the insulin/IGF-1 signalling pathway and modulation of heat shock protein expression via the enhanced nuclear translocation of transcription factor DAF-16. Additionally, both TOR and RHEB-1 are involved in the downregulation of the insulin-like peptide INS-7, an important regulator of aging. These signalling alterations resulted in increased lifespan, increased resistance to both heat and oxidative stress and a delayed onset of physiological decline associated with aging (Honjoh *et al.,* 2009).

2.5 The effect of intermittent fasting on psychological parameters

To date there has been very little research performed on the psychological effects of ADF/*m*ADF and the 5:2 diet; however there has been many studies carried out on CR and other fasting methodologies. There is a known association between fasting and mood enhancement, with patients reporting an increased sense of well-being and an easing of depression in addition to some analgesic effects in patients with chronic pain (Kerdnt *et al.*, 1982; Michalsen, 2010). This mood enhancing effect appears to be tied to increases in the production of neurotrophic factors (primarily brain derived neurotrophic factor (BDNF)) leading to increased production of opioids, serotonin and cannabinoids with the effect of reducing anxiety, depression and exhaustion (Mattson & Wan, 2005; Michalsen 2010). A Malaysian study (Hussin *et al.*, 2013) that used a religious based intermittent fasting strategy (Monday/Thursday Sunnah fast) combined with daily CR found that the participants reported decreases in anger, tension, confusion and a general improvement of mood-state, however there was no change reported in the case of depression.

While more research is required to determine if *m*ADF or 5:2 fasting are able to produce similar improvements in depressive patients, studies have demonstrated

improvements to quality of life measures and mood state with IF. Harvie *et al.* (2013) reported that there were significant improvements to mood state as assessed by profile of mood states questionnaire over a 4-month intervention period in their study of 115 overweight and obese women. Likewise, Harvie *et al.* (2011), in a study of 107 women over a year long period, reported improvements in both quality of life and mood state. Johnson *et al.* (2007) also reported improvements in energy levels and positive effects on mood state in their study investigating the effects of *m*ADF on 10 asthmatic subjects over an 8-week intervention period. While these studies suggest a potential role for IF as an adjunct to conventional treatments for depression and anxiety, more research is required.

2.6 Exercise for metabolic and cardiovascular health

Exercise has beneficial effects on cardiovascular health, weight management and general fitness. It is also involved in several other beneficial adaptions such as improved mitochondrial function, increased neurogenesis, increased insulin sensitivity, muscle mass, increased bone mineral density, decreased cancer risk and increased basal metabolism (Fentem, 1994; Neufer *et al.*, 2015). For these reasons medical professionals currently recommend 150 minutes of moderate intensity exercise per week for maintenance of good health in the adult population (Gillen & Gibala, 2014). While there is little data on long-term adherence to SIT/HIIT, the combination of a smaller time commitment and comparable results to MICT make SIT/HIIT a potential replacement for more traditional strategies. This section of the review will examine the beneficial effects attributed to exercise, with a focus on high intensity interval training and sprint interval training.

2.6.1 Metabolic benefits of exercise

While weight loss is one of the most commonly reported benefits of exercise a number of studies have shown that its effects on weight loss and adiposity are usually limited unless combined with energy restriction (Imbeault *et al.*, 1997; Swift *et al.*, 2014). Current recommendations for exercise require approximately 300 min of high intensity aerobic exercise per week to achieve clinically significant weight loss. However, it has been noted that this level of exercise commitment is neither practical nor sustainable in the long term for many people (Swift *et al.*, 2014). Given this, it would be reasonable to infer that IF might provide a convenient form of energy restriction that could be used in combination with exercise programs requiring less time commitment to provide clinically significant weight loss by assisting participants to create and maintain the negative energy balance required for such weight loss.

Exercise decreases insulin resistance in patients with impaired glucose tolerance (Hawley, 2004). This effect is believed to be mediated by improvements to mitochondrial function in skeletal muscle, though the reasons for these improvements are poorly understood. One theory is that decreasing skeletal muscle function predisposes the formation of intramycocellular lipid deposits and that this ectopic fat mass interferes with normal energy substrate utilisation. However, exercise induced increases in mitochondrial biogenesis can lead to the clearing of these fat deposits through improved fat oxidation caused by increases in respiratory chain enzyme activity (Toledo & Goodpaster, 2013). Given that weight loss through energy restriction is also able to produce improvements to insulin resistance and glucose tolerance it is likely that removal of ectopic fat stores by means other than exercise will also remove the impairment of substrate utilisation and restore normal mitochondrial function as well (Weiss *et al.*, 2006).

Babraj *et al.* (2009) reported improved insulin sensitivity in lean male participants after 2 weeks of high intensity interval training (HIIT) delivered in three sessions per week. Participants completed 4-6, 30 second sprints in each session with 4 minutes of active

rest between sprinting bouts. Although weight loss wasn't assessed during this study it demonstrates how little exercise was required per week to significantly improve glucose tolerance and insulin sensitivity.

Exercise increases neurogenesis and has a number of beneficial effects to participants including increased alertness, improved brain activity and decreased severity of anxiety-type disorders (Carro *et al.*, 2000; Trejo *et al.*, 2007). While there is still some conjecture of the processes involved, it appears to be that the bulk of these beneficial effects are governed by increased IGF-1 release/uptake in response to exercise. Carro *et al.* (2000) found that running stimulated an increase in neuronal accumulation of IGF-1, increased neuronal c-Fos and BDNF concentrations and a prolonged increase in neuronal sensitivity to afferent stimulation in Wistar rats. However, the effect of IGF-1 appears to be a local one as serum levels of IGF-1 didn't change after treadmill running and that the largest increases in the neuronal uptake of IGF-1 occurred predominantly in areas associated with motor function, metabolic control and proprioception (Carro *et al.*, 2000).

A commonly reported benefit associated with exercise is the reduction of risk factors associated with cardiovascular disease and coronary heart disease. These risk factors including elevated LDL cholesterol, elevated triglycerides, decreased HDL cholesterol, high blood pressure and metabolic syndrome which are all mitigated with regular exercise (Thompson *et al.*, 2003). In addition to these traditional markers of cardiac health there are also a number of novel indicators such as a chronic systemic anti-inflammatory response to regular physical activity along with beneficial changes to a number of haemostatic biomarkers such as fibrinogen, intracellular adhesion molecule-1 and high sensitivity C-reactive protein (Mora *et al.*, 2007). However, the mechanisms underlying these beneficial changes and their relative contributions to cardiac health remain poorly understood. Mora *et al.*, (2007) also investigated several risk factors associated with CVD/CHD and found that the data could account for

approximately 59% of the exercise related reduction in cardiovascular risk with the greatest contributions arising from alterations to haemostatic and inflammatory factors followed by blood pressure alterations, beneficial changes to blood lipid profiles and changes to body composition (BMI).

2.6.2 Physiological benefits of high intensity interval training and sprint interval training

High intensity interval training and sprint interval training are two exercise strategies that have a decreased time requirement (30-minute session with ≤ 3 min of total exercise per session) whilst maintaining similar levels of physiological benefit when compared to more traditional MICT strategies.

Gillen *et al.* (2013) examined the effects of HIIT training in 20 to 30-year-old overweight/obese women during a 6-week intervention period and reported no change in body mass but did note an increase in mitochondrial capacity along with reductions in fat mass in the legs and abdomen and an increase to fat free mass in the legs and gynoid body regions. Keating *et al.* (2014) compared SIT training to MICT on reductions in body weight in inactive, overweight men and women (*n*=38) over a 12-week intervention period and demonstrated that fat mass was reduced in the MICT group but not in the SIT group, with no significant alterations to FFM. Both groups exhibited comparable improvements to aerobic fitness.

Conversely, Trapp *et al.* (2008) examined the effects of HIIT and MICT on 45 young (~20 years), lean women and found that while cardiovascular fitness was significantly improved in the both groups only the HIIT group produced significant reductions in body weight, total fat mass, trunk fat and fasting insulin levels, with no change in adiponectin levels in either group.

This trend is supported by similar observations in children (Corte de Araujo *et al.,* 2012). They reported that body mass was significantly reduced in the HIIT group versus the MICT group in thirty obese children during a 12-week intervention period. Additionally, Corte de Araujo *et al.* (2012) noted improvements to HOMA2-IR, serum insulin levels and significant increases to VO_{2peak} in both HIIT and MICT groups.

Interval training has been associated with improvements to glycaemic control and other metabolic variables including increased mitochondrial function, fat oxidation, improvements to blood pressure and improved lipid profiles (Gibala & McGee 2008; Little *et al.*, 2011; Buchan *et al.*, 2013; Little *et al.*, 2014). Burgomaster *et al.* (2008) reported similar improvements in metabolic adaptions to both SIT and MICT during a 6-week intervention examining a total of 10 men and 10 women, reporting decreases to carbohydrate levels, increased levels of fat oxidation, increased mitochondrial activity.

While it seems unlikely that such small bouts of exercise could elicit the same effects as longer training protocols it appears that over a 24-hour period that HIIT leads to similar levels of energy expenditure when compared to MICT (Skelly *et al.*, 2014). While this study had a relatively small sample size (*n*=9) and was only performed in men it does provide some evidence for the comparable results that are observed between the two protocols. Similarly, Sevits *et al.* (2013) demonstrated that a single bout of SIT was able to increase total daily energy expenditure and RMR in a pair of studies involving 27 men (~24 years old) with a demonstrated increase in energy expenditure of ~950 kJ per day. Given an isocaloric diet this increase in energy expenditure should be sufficient to promote gradual weight loss over time. However, larger trials using both men and women would be required to confirm the validity of these results.

2.6.3 Psychological Benefits of Exercise

Recently there has been increased interest in the use of exercise in the management of numerous psychological disorders. Exercise has been demonstrated to be effective in the treatment of anorexia nervosa, major depressive disorder, bulimia nervosa, social phobia, panic disorder and PTSD (Zschucke *et al.*, 2013). While the research is compelling several limitations make it very difficult to determine the exact mechanism of these benefits. This includes the inability to monitor changes to neurotransmitter concentrations in the brain during exercise and the difficulty of separating exercise induced changes in mood state from spontaneous changes (Di Lorenzo *et al.*, 1999; Craft *et al.*, 2004).

The leading theory on the beneficial alterations in mood state with exercise is the monoamine hypothesis that was first proposed in the 1950's when it was observed that hypertensive patients that were treated with reserpine (an antihypertensive drug common in the 1950's) subsequently developed depression due to the depletion of monoamine neurotransmitters (Goldberg *et al.*, 2014). The monoamine hypothesis predicts that exercise is able to increase the production and availability of monoamine neurotransmitters (norepinephrine, serotonin & dopamine) whose production has shown to be down regulated in depression (Craft *et al.*, 2004; Goldberg *et al.*, 2014). While this increased availability of neurotransmitters appears to influence these beneficial effects, much of the evidence is currently based on plasma and urine levels of the monoamine neurotransmitters following exercise, rather than direct measurement of these levels in the brain. Thus, the observed effect might simply be a correlation rather than an actual cause. Recently it has been suggested that while monoamine neurotransmitters are important in depression, it may be that their depletion leads to the downstream dysregulation of GABA production, and that it is this dysregulation that is the source of depression rather than the monoamines themselves as GABA is the more prevalent neurotransmitter in the brain (Goldberg *et al.*, 2014).

Another popular theory is the endorphin hypothesis which states that elevated levels of β-endorphins in the brain following exercise are responsible for alterations to mood state and is a concept most often associated with the “Runner’s High” (Craft *et al.*, 2004). Similarly, this theory is largely based on raised levels of serum β-endorphins present following exercise, which may be a correlation rather than a cause as it is unknown whether increased serum endorphins can affect the brain (Craft *et al.*, 2004). In several experiments opioid antagonists have been administered to runners before exercise with differing results. Some of the studies reported no change in mood state (Markoff *et al.*, 1982; McMurray *et al.*, 1988), with another reporting that naloxone or naltrexone blocked these mood enhancing effects (Daniel & Martin, 1992). These variations have made it difficult to determine the role of endorphins in exercise induced mood enhancement given the experimental limitations.

The thermogenic theory, which suggests that temperature increases in some brain regions (particularly the brainstem) and promotes muscle relaxation and a feeling of relaxation in exercise participants has also been posited (Salerian *et al.*, 2008). The theory was originally proposed as dysregulation of temperature control has been observed in a number of psychological conditions (Salerian *et al.*, 2008). Although the research is still being performed it appears likely that these temperature changes are at least a contributing factor in exercise induced mood enhancement, particularly given that these temperature increases have potentially been implicated in the enhancement of opioid activity following exercise (Dubnov & Berry, 2013).

As well as these three main hypotheses there are also several other hypotheses that are more likely to contribute to mood enhancement in exercise. These hypotheses include the distraction hypothesis and the self-efficacy hypothesis (Craft *et al.*, 2004). The self-efficacy hypothesis suggests that exercise derived enhancements to self-worth and self-confidence may play a role in the alleviation of depression and anxiety, though there is currently little evidence to confirm or refute this (Brown *et al.*, 1992;

Craft, 2005). The distraction hypothesis is similar in that it suggests that exercise serves as a distraction from concerns, worries and anxieties and thus can promote enhanced well-being and self-image. While the distraction hypothesis is widely accepted there is little scientific evidence to verify or support it (Craft, 2005).

Another aspect relating to the use of exercise for psychological treatment is the determination of the appropriate workload to achieve maximum benefit. Di Lorenzo *et al.* (1999) used a 12-week aerobic exercise program in their study and found that in addition to increased aerobic fitness that patients experienced improvements in energy levels, improvements in the state of anxiety and depression and improvements in self-esteem. These benefits over were observed over both the short term (12 weeks) and in the long term (12 months), providing that participants maintained the exercise program (Di Lorenzo *et al.*, 1999). In contrast however, Szabo *et al.* (2013) were able to demonstrate that 3 minutes of light exercise was sufficient to enhance mood state and energy levels, which would be easily repeatable multiple times a day and would thus, be easier to maintain long term. The limitation of this study however is the lack of moderate to long-term data which would indicate whether this level of exercise would maintain its efficacy in the long term. However, Hoffman & Hoffman (2008) demonstrated that in habitual exercisers a single bout of moderate aerobic exercise (20 min) was enough to trigger increased feelings of vigour and improved mood state. Furthermore, Dunn *et al.*, (2005) were able to show that participants meeting public health guidelines for weekly aerobic exercise targets were able to achieve clinically relevant decreases in depressive symptoms. However, in contrast to Szabo, those participants who performed approximately half of the recommended exercise experienced results that were comparable to the control group in the study. While there seems to be no consensus on the appropriate “dosage” in the literature most clinicians recommend long-term exercise interventions, usually in line with public health recommendations, given the proven track record of improving mental health outcomes

in patients suffering major depressive disorder. An example of this was provided by Babyak *et al.* (2000) who demonstrated that following 6-month intervention patients undertaking aerobic exercise three times a week (40 min sessions) had significantly improved symptoms, with most participants no longer meeting the diagnostic criteria for major depressive disorder (MDD).

Ultimately given the complex nature of psychological conditions (MDD & anxiety) it's possible that most, if not all, of the current hypotheses might converge in describing the physiological changes responsible for enhancing mood state. Irrespective of the actual mode of action the consensus is that exercise has a potent beneficial effect on a number of common psychological disorders and is likely to become a more commonly prescribed intervention in the future.

2.6.4 Combined effects of CR and IF with exercise on metabolic and cardiovascular risk factors

Weight loss and improvements to both cardiovascular and metabolic risk factors are comparable in both energy restriction protocols and protocols using a combination of CER and moderate intensity exercise (CR-EX). Lefevre *et al.* (2009) examined the effects of 6 months of CR and CR-EX on body weight, CVD risk and lipid profiles in overweight, but metabolically healthy, male and female participants (*n*=36, 25-50 years). They found that both CR and CR-EX led to significant decreases in body weight, blood triglycerides, diastolic blood pressure and increases in HDL levels when compared to control (Lefevre *et al.*, 2009). While they reported no significant difference in these parameters between the two intervention groups there was a larger reduction in 10-year CVD risk in the CR-EX group due to increased aerobic capacity. Similarly, another study performed by Redman *et al.* (2007) examined the effects of CR and CR-EX on body composition and fat distribution in a cohort with a comparable sample size

and characteristics to Lefevre *et al.* (2009) over a 6-month intervention. In this study there were significant reductions in body mass in both intervention groups relative to controls. Additionally, there were significant reductions in visceral adipose tissue (VAT) and total fat mass with no difference detected between sexes. These beneficial changes were also accompanied by significant reductions in fat free mass that were apparent in both female and male participants.

Heilbronn *et al.* (2016) in a study examining the effects of CR, CR-EX and a very low-calorie diet (VLCD) on markers of metabolism, longevity and oxidative stress markers in overweight men and women (<50 years) reported similar effects on weight loss to the previous studies (with greater weight loss in the VLCD diet group) over a 6-month intervention period. They also noted significant decreases in a number of other metabolic and cardiovascular risk factors including fasting insulin levels and resting energy expenditure, with no change in oxidative stress markers and glucose levels in any intervention group. Both CR and CR-EX groups also produced significant decreases in core body temperature when compared to other groups. These studies, together with several other similar studies, have noted effects on weight loss and body composition in CR and CR-EX groups as well as reductions to ectopic fat deposits and inflammation. Collectively the aforementioned studies provide strong evidence that while CR is more effective for weight loss, both protocols are effective strategies for the reduction of cardiovascular and metabolic risk in overweight and obese populations with CR-EX producing greater reduction in total CVD risk (Larson-Meyer *et al.*, 2006; Homaee *et al.*, 2014).

While there has only been a single study conducted examining the combined effects of HIIT and IF in humans, two recent studies examining the combined effects of these protocols in animals have provided some evidence that there are synergistic effects between the two protocols on body mass and other parameters. Wilson *et al.* (2018) demonstrated that following a 10-week intervention period that body mass, lipid profiles

and body composition were improved to a greater degree in the HIIT+IF groups in comparison to IF only, HIIT only and control groups in C57BL/6 mice. Similarly, Real-Hohn *et al.* (2018) were able to demonstrate that the combination of ADF and HIIT was able to produce synergistic effects in the reduction of plasma insulin and glucose, improvements to glucose tolerance and skeletal muscle area and the prevention of weight gain over an 8-week intervention using male Wistar rats when compared to IF only, HIIT only and control groups. Taken together these results indicate there is synergism between these two protocols that might not be present between CR and HIIT individually and warrants further study.

To date, only a single study has examined the effects of *m*ADF and MICT on body weight, body composition and lipid profiles in 64 overweight or obese men and women (25-65 years) over a 12-week intervention period (Bhutani *et al.*, 2013). The results reported by Bhutani *et al.* seem to confirm the synergism noted in the aforementioned animal studies as, while there were significant decreases in body weight in all intervention groups, that there was a noticeably larger reduction in body weight in the *m*ADF + exercise group when compared to *m*ADF alone (7% vs 3% respectively). The combination group also produced greater improvements to BMI, fat mass and lipid profiles while producing similar improvements to fasting glucose when compared to the *m*ADF only group. Taken together these studies indicate the possibility of the synergism existing between other intermittent fasting and exercise protocols in humans and highlights the need for further research into this relationship to confirm the validity of these findings.

2.7 Conclusion

Intermittent fasting and SIT exhibit many parallel health benefits and it appears likely that the complementary nature of these benefits would be enhanced by combining both

strategies. Despite the potential benefits there has been very little work investigating the effects of exercise when used in combination with IF, with the bulk of the observations focussed on the fasting regime of Muslims during the Ramadan fast. While there is some indication that synergism may be present, these results aren't currently sufficient to provide direction for clinical interventions targeting MDD, metabolic syndrome, weight loss or cardiac rehabilitation.

There exists potential for improved health benefits in combining both IF and exercise strategies for the treatment of several diseases approaching epidemic levels of increase such as depression, anxiety, obesity, Type 2 diabetes and metabolic syndrome. This is important for public health programs as current weight loss strategies suffer from poor compliance, leading to unsatisfactory health outcomes for patients as well as increased medical costs for both the individual and government health agencies. The potential benefits of an exercise/IF protocol necessitate further research to determine the extent of any synergistic effects.

Chapter 3 – General Methods

3.1 Ethics/Registration

This project was approved by the Victoria University Human Research Ethics Committee (approval #: HRE 15-160) and registered with the Australian and New Zealand Clinical Trials Register (ACTRN12615001331527). There were no adverse events during the study period. This research was carried out in accordance with the principles laid out in the declaration of Helsinki (WMA, 2013)

3.2 Entry and screening

Interested participants were interviewed by phone or in person to ascertain suitability to participate in this study. Successful participants then received information outlining the study design and were given a minimum of 48 hours to read the information provided. Before entry into the study participants were required to receive medical clearance from their personal doctor.

On arrival to the first testing day participants were asked to provide informed consent and complete psychological and medical history questionnaires. Participants were then assessed for BMI and body fat percentage (by bioimpedance (Tanita Innerscan, Australia) to ensure that their values were compliant with the ranges required for the studies exclusion criteria (see below).

3.3 Recruitment and retention

An *a priori* power analysis was carried in G*power (IDRE, Germany) using data sourced from a selection of other fasting studies. From these studies the following

values were selected in consultation with a statistician: α= 0.05, effect size= 0.25 & correlation of 0.7. A small effect size was chosen to ensure that the predicted number of participants would have sufficient power to detect significant changes based on the α-value. It was also determined that based on a predicted 30% dropout rate, based on dropout rates from a number of other fasting studies with an additional 10% margin of error, (Ash *et al.*, 2003; Johnson *et al.*, 2007; Harvie *et al.*, 2011; Klempel *et al.*, 2012; Kroeger *et al.*, 2012; Harvie *et al.*, 2013; Carter *et al.*, 2016), that approximately 42 participants would need to be recruited for this study. Subsequently, forty-five overweight and obese, but otherwise healthy, sedentary males and females (BMI range 25 – 35 kg/m^2) between the ages of 18 and 45 years were subsequently recruited to participate (see Fig 3.1) using a combination of the following strategies: social media advertising, word of mouth, flyers, newspaper advertising and through the clinical trials register.

Participants were excluded based on the following criteria: unstable weight, athletic training, pregnancy, diagnosed diabetes, hypertension, body fat percentage below 22% for males and below 31% for females, use of cholesterol lowering drugs and participation in structured exercise or other exercise or diet related studies. Participants were asked to suspend other exercise training programs during the study, though recreational exercise was able to be continued as normal. Participants were required to obtain written clearance from their personal physician prior to commencing the study to clear participants of any current medical issues (i.e. diabetes, hypertension etc.). Participants were monitored throughout the study for signs of high blood pressure, cardiac anomalies or abnormal results that might constitute an adverse effect of the study on the participant's health. Finally, there was no controls in place to account for variation in menstrual cycles.

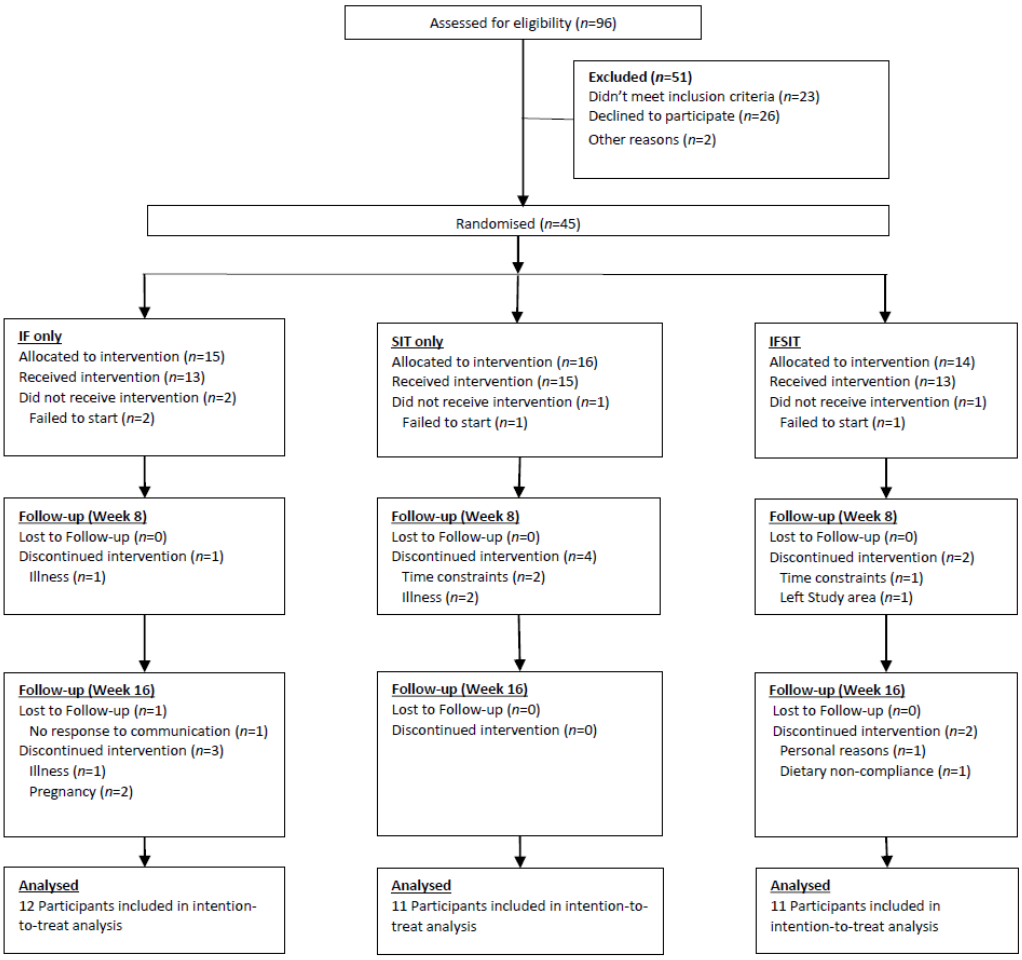


Figure 3.1 – *CONSORT diagram summarising the recruitment process, dropouts and randomisation.*

The study had an overall dropout rate of 38% with one participant being excluded for dietary non-compliance, 1 participant leaving the study due to personal reasons, 2 participant leaving due to pregnancy, 3 participants leaving due to time commitments, 1 participant leaving the study area, 4 participants leaving due to illness (unrelated to study) and with four participants failing to start the study after completing the first testing day. Participants were randomly allocated on a 1:1:1 basis (no stratification) to either 16 weeks of the 5:2 diet, 16 weeks of SIT times a week or a combination of the two protocols.

3.4 Testing schedule

Following screening and informed consent participants were issued food diaries to monitor their eating habits for one week prior to the study to ascertain basal data and were then asked to complete questionnaires to assess mood state (POMS) (McNair *et al.*, 1971), satiety (VAS), attitudes to food (VAS) (Flint *et al.*, 2000) and activity levels (IPAQ) (Booth, 2000). Hip to waist ratio was determined and a DEXA (Discovery W, Hologic Inc., USA) scan performed by a trained technician to assess body composition (fat mass, lean mass, visceral fat mass and total body fat percentage) followed by blood collection and an OGTT to assess the participant's glucose tolerance. On a separate day before commencement the participants also underwent cardiovascular analysis (SphygmoCor Xcel, AtCor Medical, Australia) followed by aerobic capacity testing (VO_{2peak}). Baseline data was collected and is presented in Table 3.1.

The above measures were repeated in the same manner in weeks 8 and 16 of the study with participants completing weekly 4-day food logs (3 non-fasting days & 1 fasting day for IF & IFSIT groups) and activity questionnaires (see appendix H). 4-day food logs were selected to monitor dietary compliance given the length of the study, as they served to provide a snapshot of each participant's weekly food intake without becoming too onerous over the study period. Every four weeks participants were required to complete psychological questionnaires (POMS & VAS) to assess mood state, satiety and attitudes to food.

Table 3.1 – *Demographic and baseline data for participants randomised into intervention groups*

	SIT	IF	IFSIT
n=	11	12	11
Age	32 ± 8.3	37 ± 5.9	39 ± 6.8
Height (cm)	167.9 ± 9.3	163.6 ± 5.5	167.1 ± 8.1
Weight (kg)	90.2 ± 20.1	81.6 ± 12.2	96.0 ± 8.1
BMI (kg/m^2)	31.7 ± 4.4	30.4 ± 3.9	33.8 ± 2.1
Hip measurement (cm)	114.4 ± 10.5	112.4 ± 7.7	118.3 ± 7.8
Waist measurement (cm)	100.9 ± 13.2	97.5 ± 12.8	105.1 ± 5.3
Visceral Fat Mass (g)	652.6 ± 255.7	615.9 ± 221.2	680.7 ± 134.7
Fat mass (kg)	33.5 ± 8.3	30.9 ± 7.9	36.1 ± 9.7
Lean Mass (kg)	52.9 ± 14.2	47.3 ± 6.2	56.2 ± 9.7
Ethnic Origin:			
ATSI	0	0	1
African	0	0	0
Arab	0	0	0
Asian	1	2	0
European	10	10	10
Other	0	0	0
Lipids:			
Total Cholesterol (mmol/L)	4.3 ± 1.0	5.0 ± 1.7	4.7 ± 1.0
Triglycerides (mmol/L)	1.1 ± 0.5	1.2 ± 0.6	1.0 ± 0.5
HDL (mmol/L)	1.4 ± 0.3	1.7 ± 0.5	1.3 ± 0.3
LDL (mmol/L)	2.6 ± 0.7	2.8 ± 1.0	2.9 ± 0.9
VO_2 peak (ml/kg.min)	27.8 ± 4.4	26.4 ± 5.4	28.7 ± 9.6
VO_2 (ml/min)	2609.5 ± 921.9	2025.4 ± 543.1	2744.5 ± 951.0
Heart Rate (bpm)	67.4 ± 8.3	67.4 ± 9.7	63.7 ± 8.9
Peripheral Systolic Blood pressure (mmHg)	130.0 ± 12.9	122.4 ± 9.5	130.1 ± 12.3
Peripheral Diastolic Blood Pressure (mmHg)	80.0 ± 7.9	75.9 ± 6.7	76.6 ± 7.3
Glucose tolerance (AUC)	779.0 ± 263.7	839.6 ± 223.3	817.2 ± 168.5
HOMA2-IR	1.6 ± 0.6	1.5 ± 0.5	1.7 ± 0.7
Fasting Glucose (mmol/L)	4.9 ± 0.6	5.0 ± 0.6	4.9 ± 0.4
HBA1c (mmol/mol)	32 ± 3.0	32 ± 3.0	30 ± 3.0

3.5 Intermittent fasting and sprint interval training protocols.

Study participants were allocated on a 1:1:1 basis to one of three 16-week protocols: IF with SIT, IF only and SIT only. Given the sample size required and initial difficulties with recruitment it was decided to use comparison groups in the place of a dedicated control group where the SIT group was used in place of a dedicated dietary control group and the IF group was used in place of an exercise control group. During

intermittent fasting, participants fasted for two non-consecutive days per weeks and ate normally (no investigator-imposed restrictions) for the other five days of the week. On fasting days men consumed a single meal of 600 kcal and women a single meal of 500 kcal, following which they were asked to fast for 24 hours with water and unsweetened black tea *ad libitum*. Dietary advice was provided in line with current nutritional guidelines (Australian Dietary Guidelines, 2013) and example recipes for 500 kcal and 600 kcal meals also provided but otherwise no specific macronutrient intake was mandated due to the free-living nature of the current study. The 5:2 diet was selected as due to its popularity in the general population as a result of mainstream marketing and its popularity in both the health and lifestyle communities. Additionally, there is currently few studies providing information relating to the efficacy and suitability of this protocol for weight loss.

The SIT protocol used was: 20 s work @ 150% VO_{2peak} (based on maximum load obtained during aerobic capacity testing) followed by 40 s of active rest @ 50 w with 4 repetitions (building to six over 4 weeks) performed three times a week. There was a three-minute warm-up and a three-minute warm down before and after the protocol respectively, performed at 50 w. All exercise sessions were performed under supervision on a magnetically braked bicycle ergometer (Lode Excalibur, Lode B.V., Netherlands). Exercise compliance, defined as the percentage of workout sessions attended was set at 90% (43/48 sessions) and any participant falling below that threshold was not included in the final analysis.

3.6 Blood Collection and Analysis

Blood was collected by IV catheterisation at least 72 hrs after the participants last bout of exercise and at least 24 hours following their previous fasting day and at least 8 hours after the participant's last meal. During catheterisation, a flexible 20 or 22-gauge

short peripheral catheter was inserted into an antecubital vein and attached to a stop valve to allow for repetitive blood sampling (0 min, 30 min, 60 min & 120 min) during the testing session. The catheter was kept patent by flushing with isotonic saline after the collection of each blood sample. The serum and plasma samples were collected using K_2EDTA, serum separator and sodium fluoride and oxalate tubes and stored at -80°C, with Dorevitch Pathology (Dorevitch Laboratories, Australia) providing analysis for standard clinical biochemistry, lipids, glucose and HBA1c. Analysis of Insulin, 8-isoprostanes, leptin, adiponectin, IL-6, high sensitivity C-reactive protein, ghrelin, TNF-α and IGF-1 were carried out by the investigators using commercially available enzyme linked immunosorbent assays (Abcam, UK and Thermo Scientific, US).

3.7 Statistical Analysis

The intention to treat data was analysed using a last observation carried forward (LOCF) manner, with the statistical analyses carried out using SPSS version 24 (IBM, USA) using an α value of 0.05. All data was analysed using repeated measures ANOVA to test for within-subject and between-subject effects. During the analysis male and female participants were pooled together in each group due to the small number of male participants present in each group (IF=1, SIT=2 & IFSIT=3). Correction for multiple pairwise comparisons was carried out using *post hoc* application of Tukey's honest significant difference (Tukey HSD) calculation. Demographic data were analysed via MANOVA to test for significant differences at baseline.

Chapter 4

The effects of sprint interval training and 5:2 diet on body composition and cardiovascular risk factors in overweight and obese individuals

4.1 Introduction

With the current global obesity epidemic, there is increasing motivation to find simple, time efficient strategies for weight reduction in overweight and obese populations. Although the role of a positive energy balance (caloric intake greater than energy expended) in the aetiology of weight gain is well understood, a large portion of the population is not following the simple recommendation to eat less and be more active (Ng *et al.*, 2014; Guthold *et al.*, 2018). Recent lifestyle-based strategies favour the use of continuous energy restriction (CER) in combination with moderate intensity continuous training (MICT) to reduce adiposity and improve cardiovascular and metabolic health. However, poor compliance, primarily due to the increased time commitment necessary for exercise, dietary planning and preparation of the food required for continuous energy restriction limits the efficacy of this approach (Sallis *et al.*, 1990; Owen *et al.*, 2004). These considerations have led researchers to investigate alternative strategies that are simpler, less time intensive, require less strict calorie counting and facilitate better long-term compliance. Two protocols gaining popularity within the health and lifestyle communities that deal with both aspects of energy balance are the 5:2 intermittent fasting diet and sprint interval training.

The 5:2 intermittent fasting diet is a strategy that consists of two non-consecutive days of fasting per week (energy intake limited to 500 Kcal for women and 600 Kcal for men), with *ad libitum* food consumption for the remainder of the week (i.e. non-fasting days) (Brown *et al.*, 2013). The 5:2 diet has been postulated by Dr Michael Mosely &

Dr Mark Mattson as an easier to follow alternative when compared to both long term CR and *m*ADF, as it only requires two days of energy restriction per week and produces improvements to cardiovascular and metabolic health similar to those of *m*ADF. These benefits include improved glycaemic control, decreased insulin levels, decreased insulin resistance, decreased basal heart rate and decreased blood pressure, improvements to blood lipid concentrations and reduction in inflammatory mediators (Harvie *et al.*, 2011; Varady, 2011; Harvie *et al.*, 2013). However, whilst direct benefits have been demonstrated with *m*ADF and other intermittent fasting strategies few studies have specifically investigated the 5:2 diet. One unintended consequence of CR is a reduction in lean mass, which may be problematic for older individuals (Weinheimer *et al.*, 2010). This effect is similarly reflected in *m*ADF (Bhutani *et al.*,2013; Trepanowski *et al.*, 2017; Gabel *et al.*, 2019), though some reports have indicated that this effect may be attenuated with IER (Varady, 2011, Varady *et al.*, 2013).

Exercise is effective at maintaining muscle mass, particularly programs involving higher exercise intensities. Combining exercise with IER could attenuate observed losses in lean mass and to improve other health related factors (Stiegler & Cunliffe, 2006). Historically, MICT has been the training methodology of choice for maximising metabolic fat oxidation potential during exercise (Romijn *et al.*, 1993). However, while MICT is effective in fat reduction, it is often subject to poor compliance due to the time required to achieve sustained results (Sallis *et al.*, 1990; CDC, 2018). MICT also provides limited stimulation of lean muscle mass in comparison to other forms of exercise due to the limited intensity range of this exercise prescription (Grgic *et al.*, 2019).

In recent years, the use of HIIT and SIT protocols as a means of achieving similar weight management results has been popularised due to its decreased time commitment, increased compliance and perceived ease of application. The reported

health benefits include improved aerobic fitness, physiological re-modelling and beneficial changes to cardiovascular and metabolic risk factors such as glucose tolerance, lipid levels, VO_{2max}, insulin levels, GLUT4 levels and blood pressure (Gibala *et al.*, 2012; Little *et al.*, 2014; Keating *et al.*, 2014; Ramirez-Jimenez *et al.*, 2017). Additionally, there is a reported association between HIIT/SIT and modest increases in fat-free mass during moderate duration studies (8-12 weeks) (Macpherson *et al.*, 2011; Gillen *et al.*, 2013). Importantly these improvements can be further enhanced through the incorporation of body weight/resistance exercises into the HIIT/SIT protocols, thereby increasing the muscular hypertrophic response and muscle mass development, leading to increased metabolic activity and enhanced maintenance of energy balance (MacInnis & Gibala, 2017). Combining HIIT/SIT with IF/CR could potentially enhance reduction of adiposity and improve lean muscle mass, resulting in other beneficial health effects. Few studies have investigated the combined effects of IF and SIT on these parameters. The current study aims to investigate the effects of these protocols (in isolation and in combination) on body composition and cardiovascular risk factors associated with obesity to test the hypothesis that the combination of SIT with IF will lead to increased fat loss (particularly in ectopic deposits), enhanced aerobic fitness and greater improvement to markers of cardiovascular and metabolic health than either SIT or IF alone.

4.2 Methods

4.2.1 Participants

Participants were recruited from the western region of Melbourne and were initially screened to determine their suitability for joining the study (please see Chapter 3 for inclusion/exclusion criteria). Volunteers who qualified were then given an information to participants form and a medical clearance form, with medical clearance required

before commencement. Upon arrival at the first testing day informed consent was obtained and the participants were assessed for body weight, BMI and body fat percentage (by bioimpedance) to ensure eligibility to participate based on the following inclusion criteria: BMI range 25-35, aged 18-45 years, non-diabetic, non-pregnant, not taking cholesterol lowering drugs, greater than 22% body fat and not participating in any other diet or exercise related studies. Participants were required to attend two testing days before commencing the study. On the first day anthropometric measurements were recorded, and body composition measurements taken, and blood collected for later analysis. On the second day SphygmoCor™ analysis of pulse wave velocity and central blood pressure were carried out along with aerobic capacity testing (VO_{2max}).

4.2.2 *Randomisation*

Based on an *a priori* power analysis (α= 0.05, effect size= 0.25 & correlation of 0.7) with a predicted 30% dropout rate it was determined that approximately 42 participants would be recruited for this study. Forty-five overweight and obese, but otherwise healthy males and females (BMI range 25 – 35 kg/m^2) between the ages of 18 and 45 years were subsequently recruited to participate (see Fig 4.1) and randomised into the intervention groups on a 1:1:1 basis in order of recruitment, with allocation modified made in attempt to match BMI, age and other baseline factors. Given the sample size required and initial difficulties with recruitment it was decided to use comparison groups in the place of a dedicated control group where the SIT group was used in place of a dedicated dietary control group and the IF group was used in place of an exercise control group.

4.2.3 *Intervention protocols*

During intermittent fasting, participants fasted for two non-consecutive days per weeks and ate normally (no investigator-imposed restrictions) for the other five days of the

week. On fasting days men consumed a single meal of 600 kcal and women a single meal of 500 kcal, following which they were asked to fast for 24 hours with water and unsweetened black tea *ad libitum*. Dietary advice was provided in line with current nutritional guidelines (Australian Dietary Guidelines, 2013) and example recipes for 500 kcal and 600 kcal meals also provided but otherwise no specific macronutrient intake was mandated due to the free-living nature of the current study. The 5:2 diet was selected as due to its popularity in the general population as a result of mainstream marketing and its popularity in both the health and lifestyle communities. Additionally, there is currently few studies providing information relating to the efficacy and suitability of this protocol for weight loss.

The SIT protocol used was: 20 s work @ 150% VO_{2peak} (based on maximum load obtained during aerobic capacity testing) followed by 40 s of active rest @ 50 w with 4 repetitions (building to six over 4 weeks) performed three times a week. There was a three-minute warm-up and a three-minute warm down before and after the protocol respectively, performed at 50 w. All exercise sessions were performed under supervision on a magnetically braked bicycle ergometer (Lode Excalibur, Lode B.V., Netherlands). Exercise compliance, defined as the percentage of workout sessions attended was set at 90% (43/48 sessions) and any participant falling below that threshold was not included in the final analysis.

4.2.4 Body Weight, Height & Body Mass index

Participants presented to the laboratory well hydrated following an overnight fast (minimum 8 hours). After voiding their bladder, heights were measured using a wall mounted stadiometer (S+M, Medshop, Australia) and their body weight determined using a bioimpedance scale (Innerscan BC-545, Tanita Corporation, Japan). Body mass index was then calculated using the following equation: BMI = Weight in Kg/Height$(m)^2$.

4.2.5 Hip Waist Ratio

Waist measurements were taken using a flexible measuring tape at the mid-point between the topmost surface of the iliac crest and last rib and hip measurements were taken around the fullest portion of the buttocks. Each measurement was repeated twice, and an average value taken. Hip to waist ratio was determined using the following formula: HWR = Waist measurement (cm)/ Hip measurement (cm).

4.2.6 Body Composition (DEXA, IMAT)

Body composition was assessed using a full body DEXA scan (Discovery W, Hologic Inc., USA). Prior to scanning participants were instructed to void their bladder, to remove all metal objects and change into light clothing. The participants were then asked to lie face up on the scanner table with their hands palm down, fingers spread, and their feet arranged with the toes pointing inwards as per the manufacturer's instructions. Once in the correct position the participant's feet were secured in position using tape to prevent unnecessary movement. Measurements for body fat percentage, visceral fat mass, lean mass and fat mass were recorded for each participant. All scans were performed by a trained technician.

Intramuscular fat area was determined using peripheral quantitative computed tomography (pqCT) (XCT-3000, Stratec Medizintechnik GmbH, Germany). Participants were asked to remove all jewellery from their hands and forearm, following which their forearm length was determined for their non-dominant arm using a ruler to measure the distance between the point of the elbow and the styloid process of the ulna. The participant was then seated comfortably with their non-dominant arm placed into the scanner, which was secured to the hand rest to eliminate any unintended movement. The scanner was then positioned so that the targeting laser was orientated a few millimetres distal to the styloid process of the radius. A scout scan was then

performed to locate the carpal articular surface as the starting point for the scanning procedure, after which scans were taken at 4% and 66% of forearm length.

Analysis was carried out on the scan performed at 66% of forearm length using Stratec software version 6.20C (Stratec Medizintechnik GmbH, Germany) to determine intramuscular fat area. First a region of interest was defined to exclude subcutaneous fat, then density thresholds of 1 mg/cm^3 and 60 mg/cm^3 were used to isolate fat content within the region of interest. Intramuscular fat area was determined by subtracting the area measurements taken from the 60 mg/cm^3 from the area measurement taken at 1 mg/cm^3.

4.2.7 SphygmoCor Analysis

4.2.7.1 Central Blood Pressure Estimation

Prior to assessment the participant was asked to abstain from fasting and caffeinated beverages on the morning of the appointment, but otherwise had no investigator imposed restrictions. Participants were then asked to lie comfortably on an examination table and were fitted with pneumatic blood pressure cuffs on both their upper arm and thigh. They were then asked to lie still for 10 minutes without crossing their legs, talking or making extraneous movements to allow their blood pressure to settle to resting values. Peripheral and central blood pressures were then assessed automatically using the SphygmoCor Xcel analyser (AtCor Medical, Australia).

4.2.7.2 Pulse Wave Velocity Assessment

To determine pulse wave velocity the location of the carotid pulse was first identified and marked. Measurements were then taken from the carotid pulse to the sternal

notch, from the sternal notch to the thigh cuff and from the femoral pulse to the thigh cuff using a flexible measuring tape. These measurements were then entered into the software (XCEL version 1.2.0.7, AtCor Medical, Australia) to provide the pulse wave distance. A tonometer was then placed over the carotid pulse until a steady pulse measurement was registered by the software, causing the thigh cuff to inflate automatically. The software then compared the pulse measurements to estimate the pulse wave velocity (m/s).

4.2.8 Aerobic Capacity (VO_2) Testing

Participants were asked to abstain from eating for an hour prior to testing and were asked to dress for exercise. A graded exercise test using a ramped workload protocol was then performed on a magnetically braked cycle ergometer (Lode Excalibur, Lode B.V., Netherlands). The participant was fitted with a heart rate monitor (Polar V800, Polar, Finland), nose clamp and mouthpiece (Hans Rudolph, USA) prior to commencing the test. The participant was then asked to sit for 10 minutes to allow respiratory gas values to stabilise.

Male participants began at a power output of 50 watts for three minutes after which the workload was increased incrementally by 50 w every 3 minutes until 9 minutes, after which the load increased in 25 w increments each minute until volitional fatigue. The female protocol varied in that the starting load was 25 w and that all subsequent increases in load were 25 w. Oxygen uptake (VO_2) and respiratory exchange ratio (RER) were measured every 30 seconds using the Moxus Metabolic System (AEI technologies, USA). Due to the fitness level of participants VO_{2peak} was determined to have been achieved upon volitional fatigue or if two of the following criteria were met: a respiratory exchange ratio ≥ 1.15, an RPE score ≥ 19 and/or a peak heart rate within ± 10 beats of the participant's age predicted maximum (determined as: 220 - age). Heart

rate was determined and continuously monitored using a heart rate monitor with chest strap and wristwatch (Polar V800, Polar, Finland) and perceived exertion (Borg scale rating of perceived exertion) was measured during each interval. VO_{2peak} assessment was carried out at baseline, week 8 and week 16.

4.2.9 Serum and Plasma markers of cardiovascular risk

Blood was collected by IV catheterisation as per the procedures outlined in Chapter 3. Analysis of total cholesterol, HDL, LDL and TG were performed commercially (Dorevitch Laboratories, Australia).

4.2.10 Nutritional Data

Participant's nutritional data was collected by either a 4-day Food Log (see Appendix H) or using either MyFitnessPal™ or My Net Diary™. Participants were asked to record four days of food intake including portion sizes (using provided instructions to estimate), with those in fasting groups asked to record one of their fasting days to assess dietary compliance. Food diaries were analysed using Foodworks 9 software (Xyris Software, Australia). Dietary intake was assessed for macronutrient content, total energy and fasting energy as determined by AusBrands™ and AusFood™ databases within Foodworks. When portion sizes weren't specified, they were estimated from other diary entries or treated as single recommended serves for each component of the meal.

4.2.11 Statistical analysis

The intention to treat data was analysed using a last observation carried forward (LOCF) manner, with the statistical analyses carried out using SPSS version 24 (IBM, USA) using an α value of 0.05. All data was analysed using repeated measures ANOVA to test for within-subject and between-subject effects. During the analysis male and female participants were pooled together in each group due to the small number of male participants present in each group (IF=1, SIT=2 & IFSIT=3). Correction for multiple pairwise comparisons was carried out using *post hoc* application of Tukey's honest significant difference (Tukey HSD) calculation. Demographic data were analysed via MANOVA to test for significant differences at baseline.

4.3 Results

4.3.1 Recruitment and retention

Forty-five participants were recruited for this study. An overall dropout rate of 38% was observed with one participant being excluded for dietary non-compliance, one participant leaving the study due to personal reasons, two participants leaving due to pregnancy, 3 participants leaving due to time restrictions, one participant relocated to another city, four participants left due to illness (unrelated to study) and with four participants failing to start the study after completing baseline testing (Fig. 4.1). A total of 28 participants completed the entire study (IF=8, IFSIT=11, SIT=9) with a further 6 participants included in the intention to treat analysis (IF=4, IFSIT=2, SIT=0) with a total of 34 participants included in the final analysis.

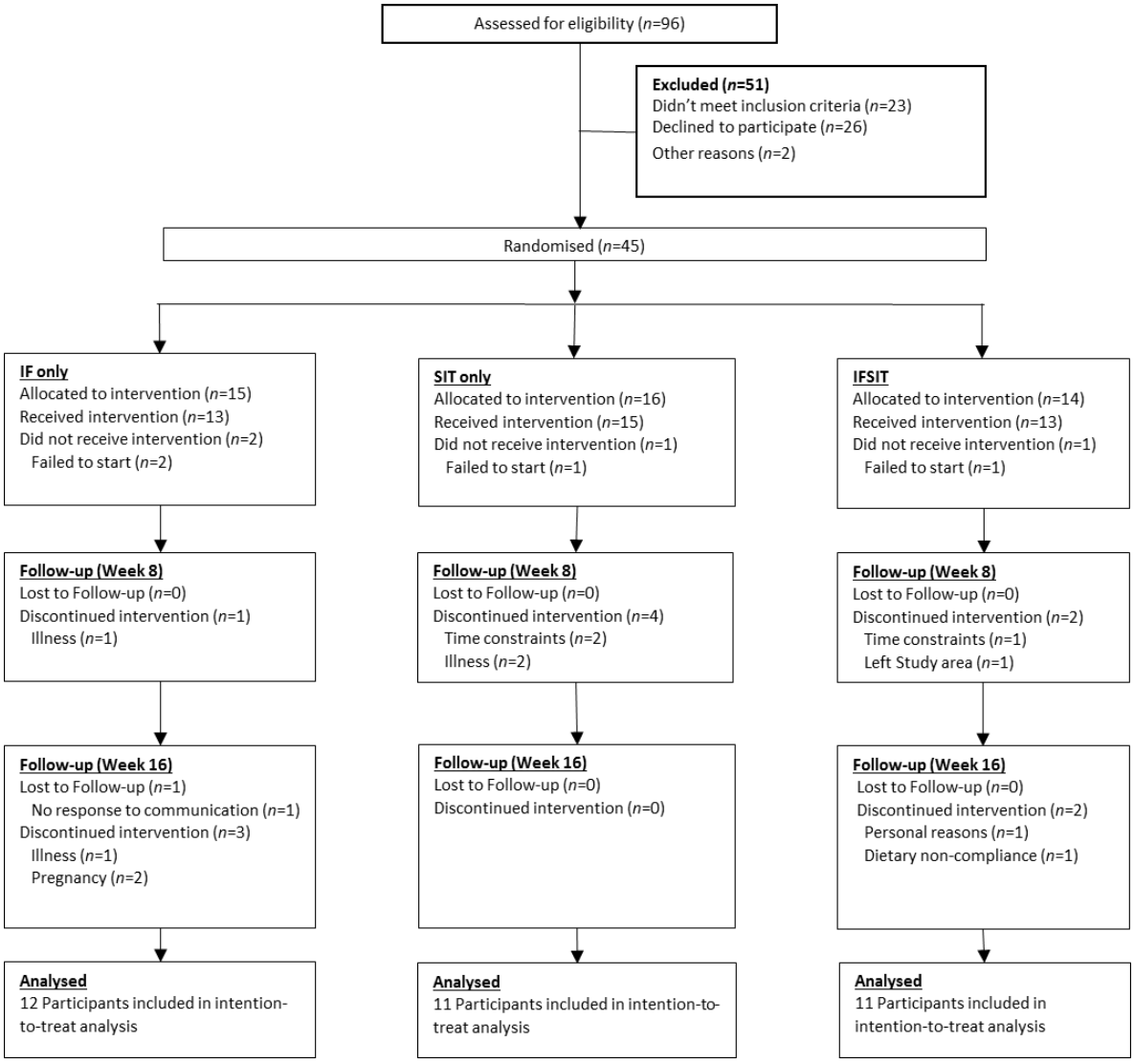


Figure 4.1 – *CONSORT diagram summarising the recruitment process, dropouts and randomisation.*

4.3.2 Pre-intervention anthropometric, metabolic, blood and cardiovascular measures

The baseline demographic data for the 34 participants who completed the 16-week intervention period and/or were included in the intention to treat analysis is detailed in Table 4.1. Statistical analysis revealed no significant difference between groups at baseline in age (p=0.081), height (p=0.422), body weight (p=0.066), BMI (p=0.105), fat mass (p=0.360), lean mass (p=0.130), intramuscular adipose tissue (IMAT) (p=0.934),

total cholesterol (p=0.413), triglycerides (p=0.617), HDL cholesterol (p=0.124), LDL cholesterol (p=0.702). Additionally, there were no significant differences detected in the following cardiovascular parameters: Pulse wave velocity (p=0.128), heart rate (p=0.533), peripheral blood pressure (Systolic: p=0.221, Diastolic: p=0.388), central blood pressure (Systolic: p=0.403, Diastolic: p=0.443), glucose tolerance measured as area under curve (p=0.771), HOMA2-IR (p=0.489), fasting glucose (p=0.895) and HBA1c (p=0.353). There was, however, a significant difference detected in VO_{2peak} (p=0.036) and near significant differences in body weight (p=0.066), waist measurements (p=0.068), absolute VO_2 (p=0.096) and age (p=0.081).

Table 4.1 – *Baseline and demographic data for the study participants. Values are given ± SD.*

	SIT	IF	IFSIT
n=	11	12	11
Age	32 ± 8.3	37 ± 5.9	39 ± 6.8
Height (cm)	167.9 ± 9.3	163.6 ± 5.5	167.1 ± 8.1
Weight (kg)	90.2 ± 20.1	81.6 ± 12.2	96.0 ± 8.1
BMI (kg/m^2)	31.7 ± 4.4	30.4 ± 3.9	33.8 ± 2.1
Hip measurement (cm)	114.4 ± 10.5	112.4 ± 7.7	118.3 ± 7.8
Waist measurement (cm)	100.9 ± 13.2	97.5 ± 12.8	105.1 ± 5.3
Visceral Fat Mass (g)	652.6 ± 255.7	615.9 ± 221.2	680.7 ± 134.7
Fat mass (kg)	33.5 ± 8.3	30.9 ± 7.9	36.1 ± 9.7
Lean Mass (kg)	52.9 ± 14.2	47.3 ± 6.2	56.2 ± 9.7
Ethnic Origin:			
ATSI	0	0	1
African	0	0	0
Arab	0	0	0
Asian	1	2	0
European	10	10	10
Other	0	0	0
Lipids:			
Total Cholesterol (mmol/L)	4.3 ± 1.0	5.0 ± 1.7	4.7 ± 1.0
Triglycerides (mmol/L)	1.1 ± 0.5	1.2 ± 0.6	1.0 ± 0.5
HDL (mmol/L)	1.4 ± 0.3	1.7 ± 0.5	1.3 ± 0.3
LDL (mmol/L)	2.6 ± 0.7	2.8 ± 1.0	2.9 ± 0.9
VO_2 peak (ml/kg.min)	27.8 ± 4.4	26.4 ± 5.4	28.7 ± 9.6
VO_2 (ml/min)	2609.5 ± 921.9	2025.4 ± 543.1	2744.5 ± 951.0
Heart Rate (bpm)	67.4 ± 8.3	67.4 ± 9.7	63.7 ± 8.9
Peripheral Systolic Blood pressure (mmHg)	130.0 ± 12.9	122.4 ± 9.5	130.1 ± 12.3
Peripheral Diastolic Blood Pressure (mmHg)	80.0 ± 7.9	75.9 ± 6.7	76.6 ± 7.3
Glucose tolerance (AUC)	779.0 ± 263.7	839.6 ± 223.3	817.2 ± 168.5
HOMA2-IR	1.6 ± 0.6	1.5 ± 0.5	1.7 ± 0.7
Fasting Glucose (mmol/L)	4.9 ± 0.6	5.0 ± 0.6	4.9 ± 0.4
HBA1c (mmol/mol)	32 ± 3.0	32 ± 3.0	30 ± 3.0

4.3.3 Intervention anthropometric measures

4.3.3.1 Body weight analysis

Significant reductions in body weight were observed between both fasting groups (IF+IFSIT) over 16 weeks with a greater decrease observed in the IF group, while body weight increased in the SIT group (Fig. 4.2). There was no significant difference identified between the fasting groups. Repeated measures ANOVA analysis for within-

subject and between-subject effects revealed main effects for time (p=0.000), group (p=0.005) and group*time (p=0.004) (Table A4.1). With *post hoc* multiple comparisons analysis revealing significant interaction effects between the SIT and IF groups (p=0.024) and between the SIT and IFSIT group (p=0.015).

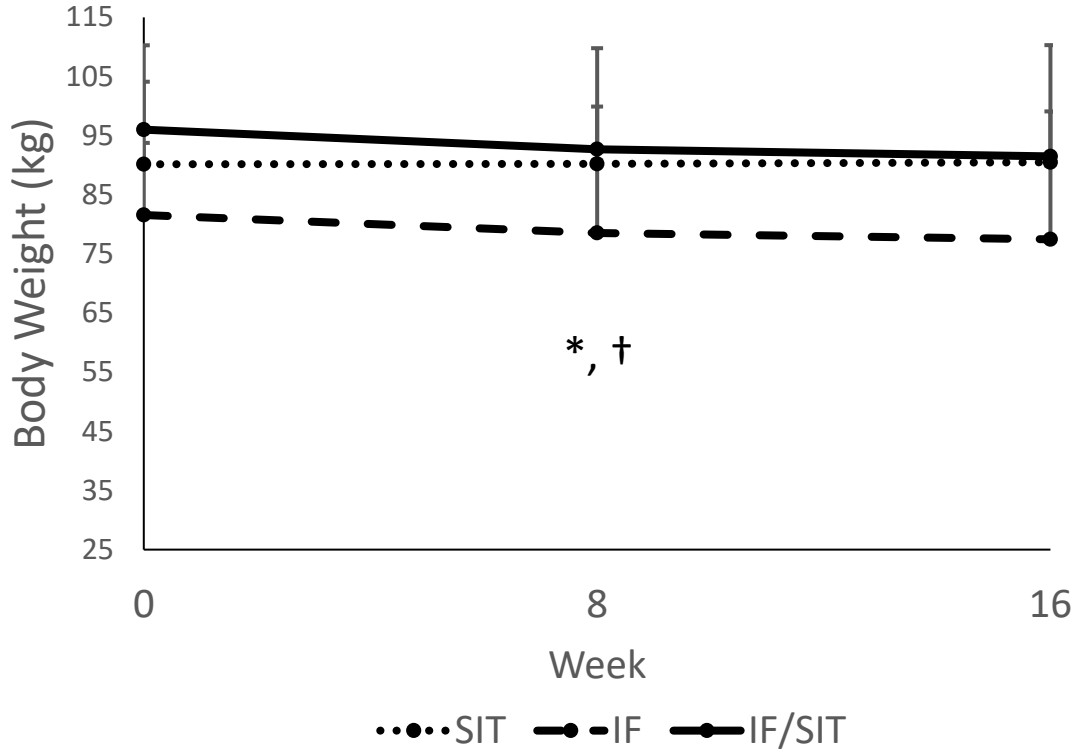


Figure 4.2 – *Mean changes in body weight over a 16-week intervention period. Data points represent week 0, 8 & 16. Error bars represent ± S.D. (*) denotes a significant main effect (p<0.05) between SIT and IF groups, (†) denotes a significant main effect between SIT and IFSIT groups.*

Similarly, BMI reflected the body weight trends with the fasting groups producing greater reductions in BMI relative to the SIT only group following the 16-week

intervention period (Fig. 4.3) with comparable decreases in both the IF and IFSIT groups. Repeated measures ANOVA analysis revealed main effects for time (p=0.000), group (p=0.004) and group*time (p=0.002) (Table A4.1), with multiple comparisons analysis indicating significant interaction effects between the SIT and IF groups (p=0.011) and between the SIT and IFSIT group (p=0.008). These changes amount to 7.9% and 6.1% decreases in BMI in the IF and IFSIT cohorts over the course of the intervention while participants in the exercise only group experienced a 0.5% increase in BMI overall.

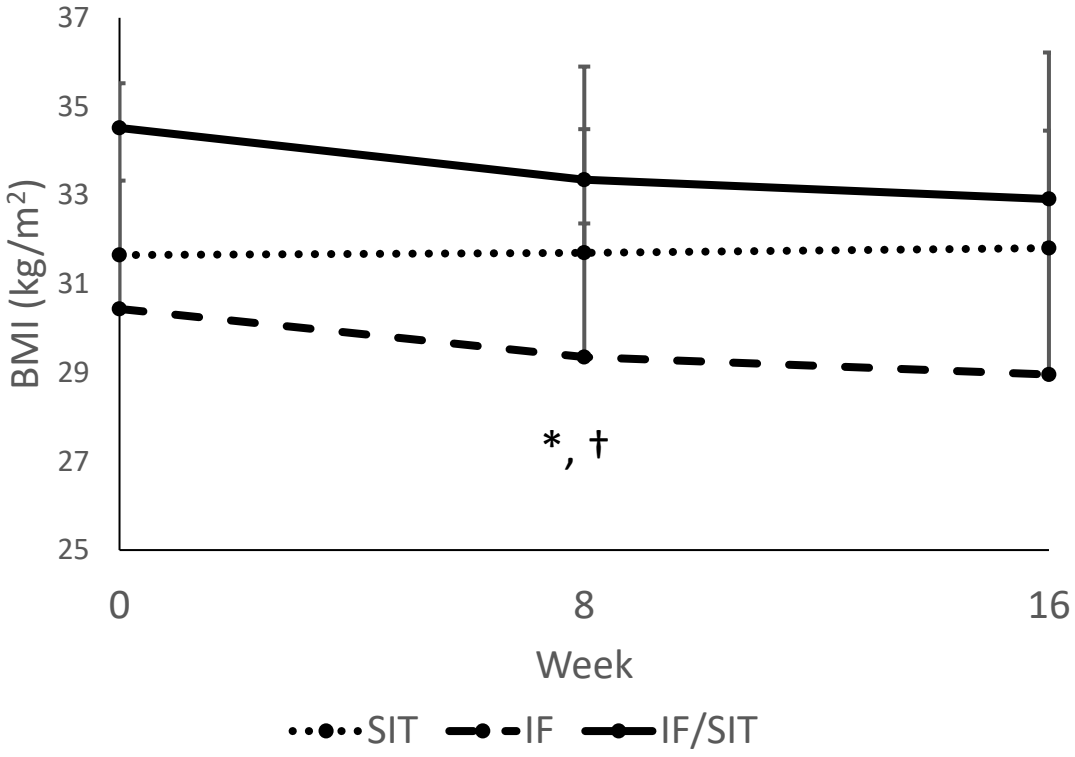


Figure 4.3 – *Mean changes in body mass index over a 16-week intervention period. Data points represent week 0, 8 & 16. Error bars represent ± S.D. (*) denotes a significant main effect (p<0.05) between SIT and IF groups, (†) denotes a significant main effect between SIT and IFSIT groups.*

4.3.3.2 Body composition

Fat mass trends paralleled those of body mass for the intervention groups (Fig. 4.4) with similar reductions in the IF and IFSIT groups. Most the change observed in the fasting groups appeared to be due to reductions in fat mass rather than lean mass. Within-subject and between-subject analysis revealed significant main effects for time (p=0.008), group (p=0.008) and group*time (p=0.008) (Table A4.1). Additionally, multiple comparisons analysis indicated significant interaction effects between both the SIT and IF groups (p=0.019) and between the SIT and IFSIT groups (p=0.035).

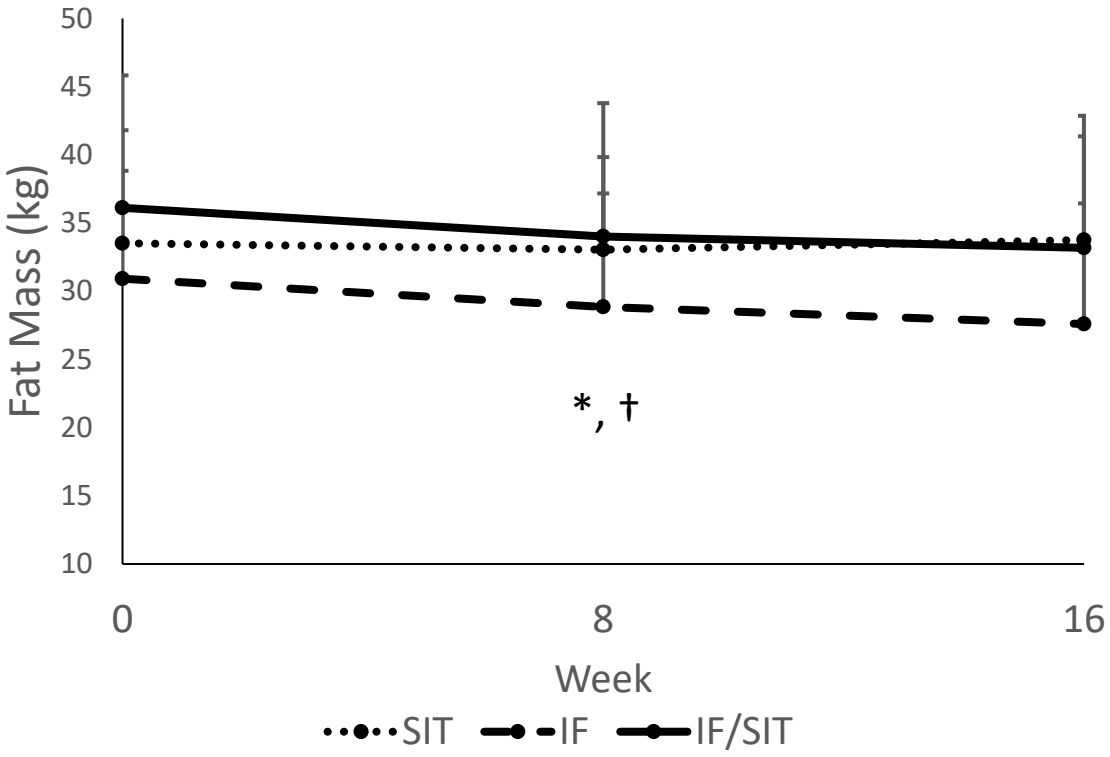


Figure 4.4 – *Mean changes in fat mass over a 16-week intervention period. Data points represent week 0, 8 & 16. Error bars represent ± S.D. (*) denotes a*

significant main effect (p<0.05) between SIT and IF groups and (†) denotes a significant main effect between SIT and IFSIT groups.

Regional fat mass distribution after 16 weeks reflected whole body changes (Fig. 4.5). Body regions showed relatively uniform fat losses, with greater variance recorded in the trunk region relative to other segments of the body.

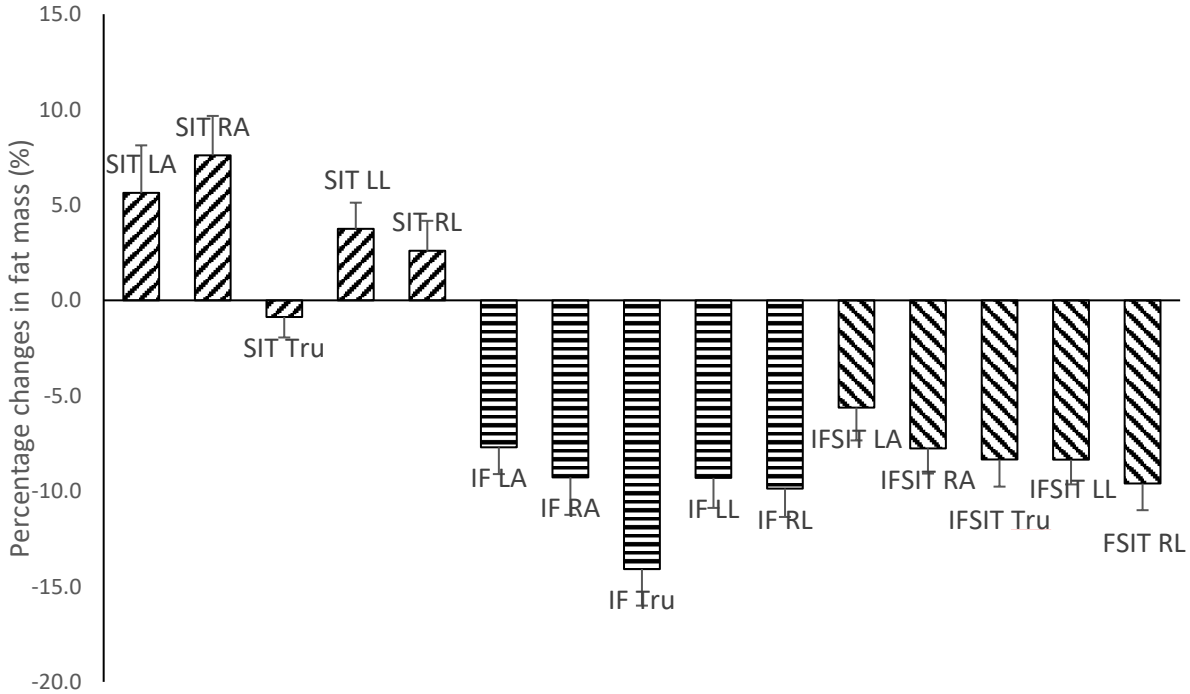


Figure 4.5 – *Percentage changes in fat mass averages in the left arm (LA), right arm (RA), trunk (Tru) and left leg (LL) and right leg (RL) over a 16 week intervention period in participants undertaking 16 weeks of either intermittent fasting, sprint interval training or a combination of the two.*

Following the 16-week intervention period there were no significant main effects for time, group or group*time (Table A4.1) for lean mass, there were however, strong trends for group and group*time effects (p=0.057 & p=0.054) identified. Multiple comparisons analysis also indicated a strong but non-significant difference between the IFSIT and SIT groups (p=0.051). Analysis of lean mass by body region (Fig. 4.7) demonstrated that the SIT only group increased in lean mass in all body regions except the trunk, while the fasting groups showed reductions in all body regions, with increased magnitude in the IFSIT group.

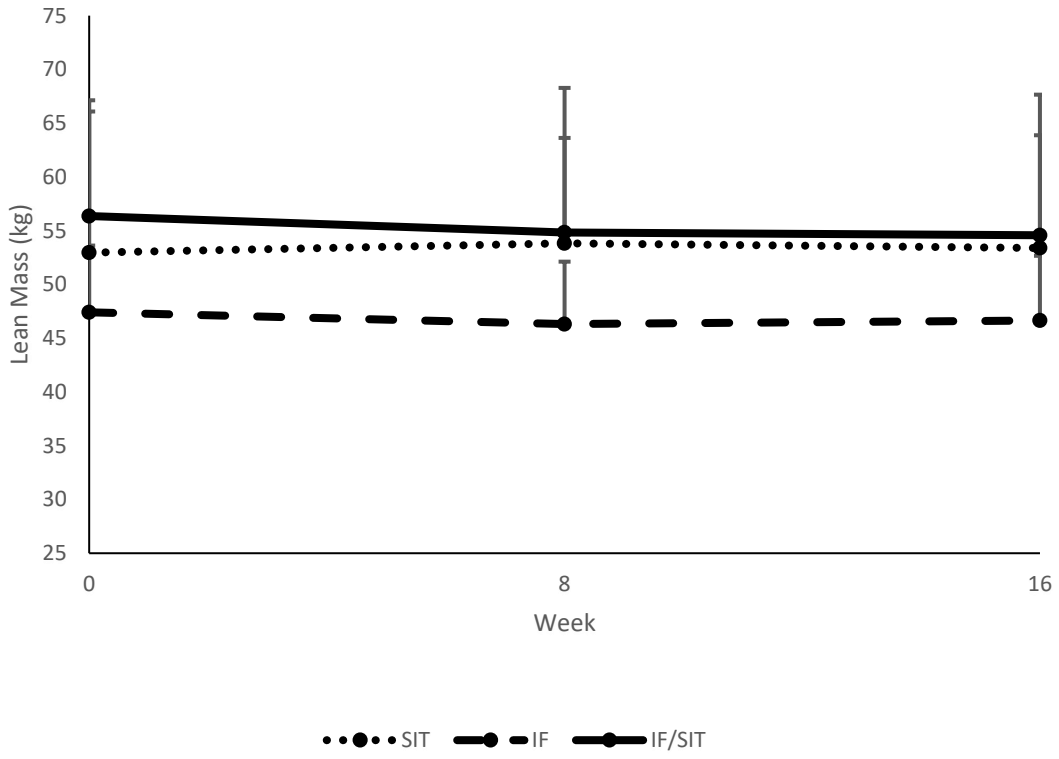


Figure 4.6 – *Mean changes in lean mass over a 16-week intervention period. Data points represent week 0, 8 & 16. Error bars represent ± S.D.*

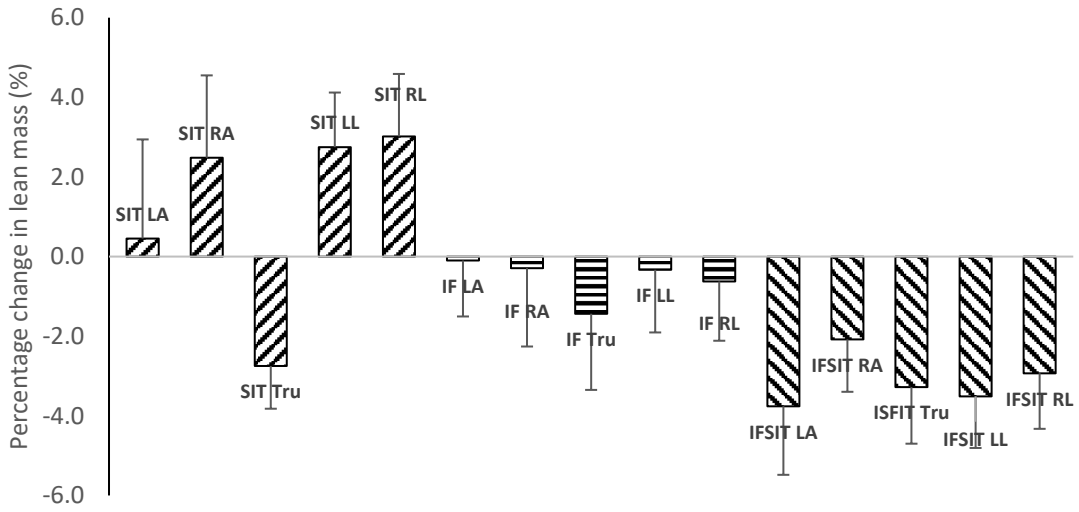


Figure 4.7 – *Percentage changes in lean mass averages in the left arm (LA), right arm (RA), trunk (Tru) and left leg (LL) and right leg (RL) over a 16 week intervention period in participants undertaking 16 weeks of either intermittent fasting, sprint interval training or a combination of the two.*

Shown in Table 4.2 are the results for visceral fat mass (VAT), intramuscular fat mass (IMAT) and hip to waist ratio (HWR) measured at 0, 8 and 16 weeks. Following analysis there was a significant effect for time noted in VAT mass (p=0.001) (Table A4.1) but no significant main effects or interactions otherwise. All groups demonstrated decreases to VAT with IF demonstrating the largest decrease, followed by IFSIT and SIT.

IMAT analysis revealed no significant main effects or interactions for time, group, or group*time (Table A4.1). Additionally, there were no significant interactions between groups. Analysis of HWR revealed a significant main effect for time (p=0.022) (Table A4.1) but no other significant effects or interactions otherwise. IMAT decreased in both fasting groups and increased in the SIT group, with the largest decrease appearing in the IF group.

Table 4.2 – *Changes to cardiovascular and body composition measures*

	SIT			IF			IFSIT		
Week	Week 0	Week 8	Week 16	Week 0	Week 8	Week 16	Week 0	Week 8	Week 16
VAT (g)	652 ± 256	638 ± 233	649 ± 267	615 ± 221	552 ± 247	525 ± 213	680 ± 135	637 ± 147	602 ± 153
IMAT (mm^2)	92 ± 40	97 ± 20	98 ± 39	90 ± 27	88 ± 15	82 ± 13	82 ± 28	82 ± 23	80 ± 23
Hip:Waist ratio	0.88 ± 0.07	0.86 ± 0.07	0.87 ± 0.08	0.87 ± 0.08	0.87 ± 0.07	0.86 ± 0.07	0.89 ± 0.07	0.84 ± 0.08	0.84 ± 0.08
Waist circumference (cm)	100 ± 13	98 ± 11	99 ± 13	97 ± 13	96 ± 11	94 ± 10	105 ± 8	98 ± 12	96 ± 12
PBP Systolic (mmHg)	130 ± 13	128 ± 13	127 ± 13	122 ± 9	121 ± 8	123 ± 8	130 ± 12	132 ± 9	134 ± 12
PBP Diastolic (mmHg)	80 ± 8	78 ± 10	77 ± 10	76 ± 7	74 ± 6	77 ± 7	77 ± 7	78 ± 4	80 ± 6
CBP Systolic (mmHg)	115 ± 11	112 ± 11	116 ± 15	110 ± 7	109 ± 7	111 ± 7	116 ± 11	118 ± 7	120 ± 11
CBP Diastolic (mmHg)	81 ± 8	78 ± 10	78 ± 11	77 ± 7	75 ± 6	77 ± 7	78 ± 7	79 ± 4	81 ± 6
Heart Rate (bpm)	67 ± 8	64 ± 8	63 ± 13	68 ± 10	63 ± 10	65 ± 10	64 ± 9	63 ± 8	61 ± 6
Pulse wave velocity (m/s)	6.1 ± 1.0	6.1 ± 1.1	6.3 ± 1.4	5.8 ± 0.9	5.7 ± 0.8	5.7 ± 0.8	6.6 ± 0.7	6.8 ± 0.9	6.6 ± 0.9
Total Cholesterol (mmol/L)	4.5 ± 1	4.6 ± 1.1	4.4 ± 0.9	4.7 ± 1.7	4.7 ± 2.0	4.6 ± 1.9	4.7 ± 1.0	4.6 ± 0.9	4.4 ± 1.0
Triglycerides (mmol/L)	1.0 ± 0.5	1.2 ± 0.7	1.1 ± 0.6	1.2 ± 0.6	1.0 ± 0.6	1.1 ± 0.6	1.0 ± 0.5	0.9 ± 0.3	0.9 ± 0.3
LDL Cholesterol (mmol/L)	2.5 ± 0.7	2.5 ± 0.8	2.4 ± 0.7	2.8 ± 1.0	2.8 ± 0.5	2.7 ± 1.3	2.9 ± 0.9	2.9 ± 0.8	2.8 ± 0.9
HDL Cholesterol (mmol/L)	1.4 ± 0.3	1.5 ± 0.4	1.5 ± 0.4	1.7 ± 0.5	1.6 ± 0.5	1.7 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3

Abbreviations: PBP: Peripheral Blood Pressure, LDL: Low Density Lipoprotein HDL: High Density Lipoprotein: VAT: Visceral Adipose Tissue

4.3.4 Cardiovascular risk factors

Table 4.2 outlines the changes to cardiovascular measurements over the 16-week intervention period. Following analysis of these results by repeated measures ANOVA there were no significant main effects detected for group or group*time for either peripheral blood pressure or central diastolic pressure measurements, however there were significant effects for time identified for both central (p=0.016) and peripheral (p=0.012) systolic blood pressure (Table A4.2). Additionally, there were no significant interactions effects detected between the three intervention groups for central or peripheral blood pressure measurements with comparable decreases occurring in each group.

Changes to heart rate were recorded in participants completing 16 weeks of either IF, SIT or IFSIT and were subsequently analysed by repeated measures ANOVA. Within-subject and between-subject analysis revealed a significant main effect of time (p=0.020) with all groups recording decreased values over the intervention period (Table A4.2) but no significant effects for group, group*time or between groups analysis. Heart rate decreased to the greatest extent in the SIT group (-4.1 bpm), with comparable decreases in both the IF (-2.3 bpm) and IFSIT (-2.4 bpm) groups. Analysis

of pulse wave velocity (Table A4.2) results revealed no significant effects over the 16-week intervention period, with little change in any group.

Figure 4.8 contains the VO_{2peak} results for each of the intervention groups at all three time points with greatest increase evident in the IFSIT group. Between-subject and within-subject effects analysis revealed significant main effects of time (p=0.000), group (p=0.001) and group*time (p=0.002) (Table A4.2). Multiple comparisons analysis detected significant interactions between the intermittent fasting and combined groups (p=0.001) but no significant differences between the SIT and IF groups (p=0.151) or the SIT and IFSIT groups (p=0.097).

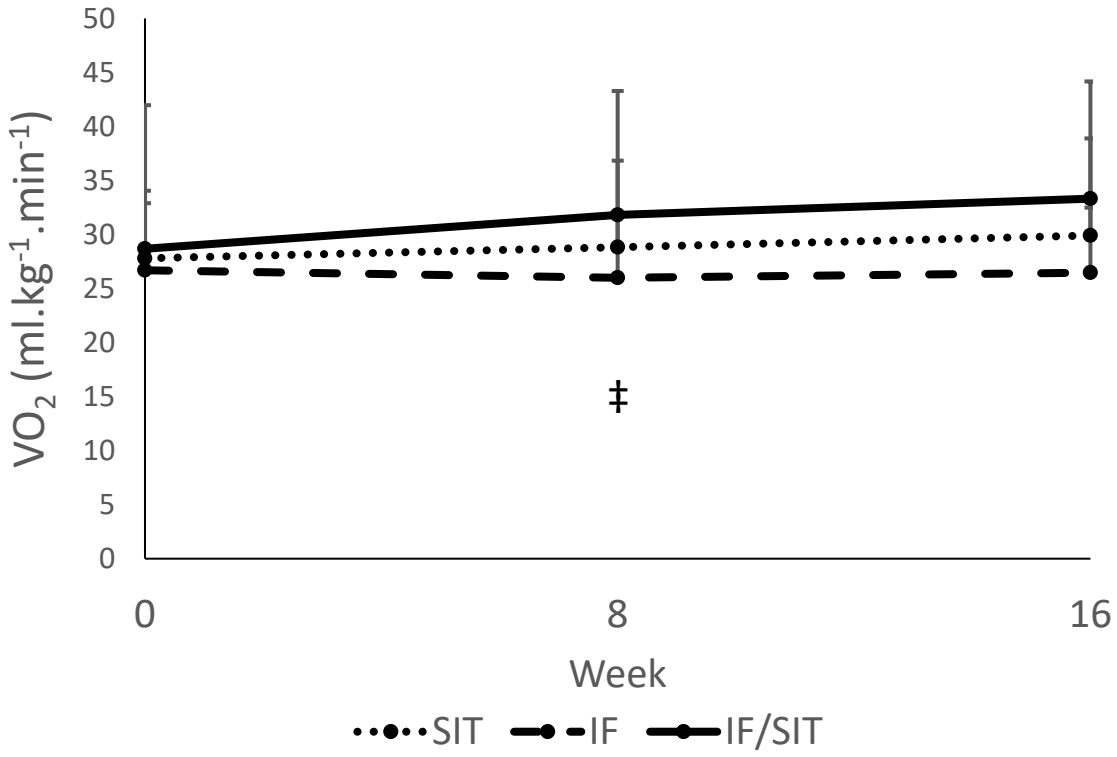


Figure 4.8 – *Mean changes in VO_{2peak} over a 16-week intervention period. Data points represent week 0, 8 & 16. Error bars represent ± S.D.* (‡) *denotes a significant main effect ($p<0.05$) between IF and IFSIT groups.*

Represented in figure 4.9 are the data for absolute VO_2, with the largest increase recorded for the IFSIT group when compared to the SIT group. After analysis there was no significant main effects for time, however there were significant effects for group (p=0.012) and group*time (p=0.009) recorded (Table A4.2) with significant differences recorded between the IF and IFSIT (p=0.009) groups.

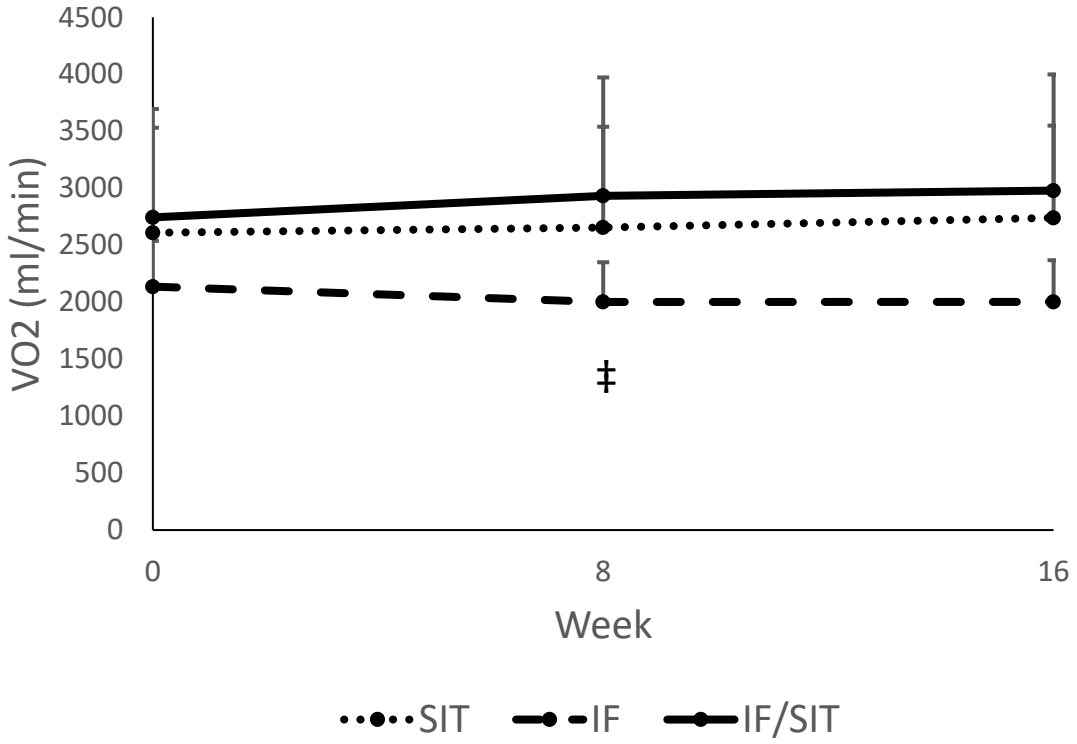


Figure 4.9 – *Mean changes in Absolute VO_2 measurements over a 16-week intervention period. Data points represent week 0, 8 & 16. Error bars represent ± S.D.* (‡) *denotes a significant main effect between IF and IFSIT groups.*

Shown in figure 4.10 are the data for VO_2 when factored for lean muscle mass with the largest increase recorded for the IFSIT group when compared to the SIT group. After within-subject and between-subject analysis there were significant effects for time (p=0.045), group (p=0.001) and group*time (p=0.009) noted (Table A4.2), with a

significant interaction effect between the IF & IFSIT groups (p=0.001) and a near significant trend towards a difference between the SIT and IFSIT groups (p=0.073).

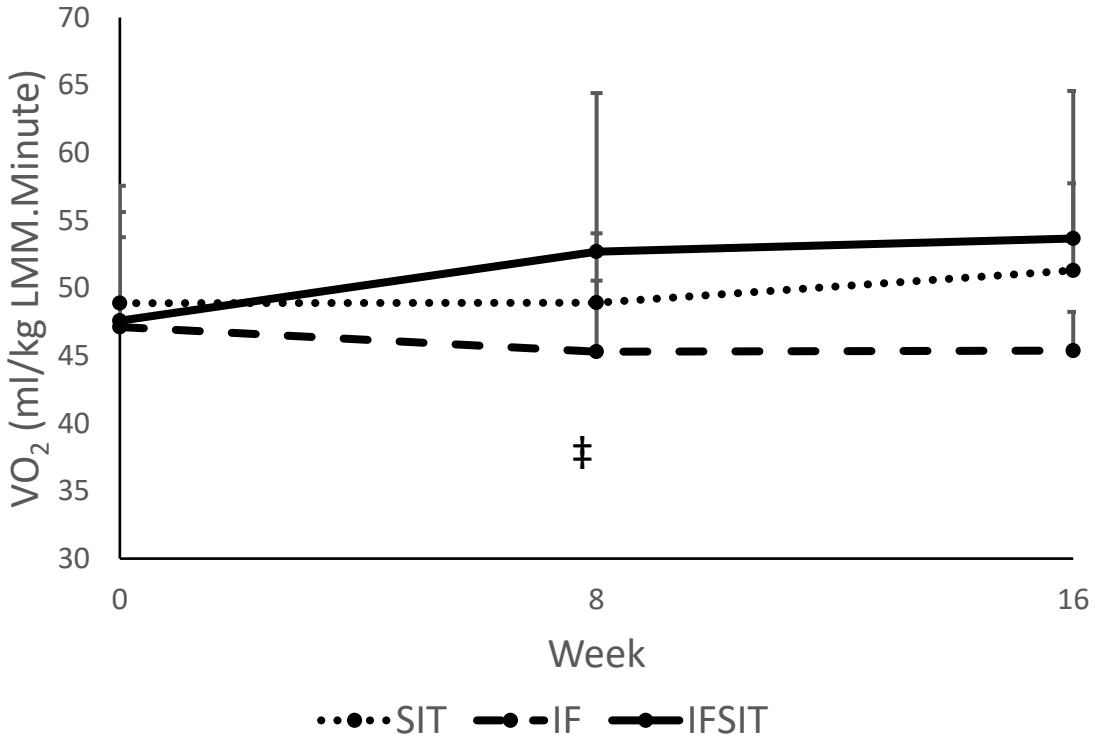


Figure 4.10 – *Mean changes in VO_2 measurements when compared to lean muscle mass over a 16-week intervention period. Data points represent week 0, 8 & 16. Error bars represent ± S.D.* (‡) *denotes a significant main effect ($p<0.05$) between IF and IFSIT groups.*

Lipid profiles were measured in participants undertaking 16 weeks of either the 5:2 diet, sprint interval training or a combination of both (Table 4.2). Analysis of between-subject and within-subject effects by repeated measures ANOVA revealed no significant main effects of time, group or group*time (Table A4.2) for total cholesterol. Similarly, there were no significant main effects for time, group or group*time noted for triglyceride levels (Table A4.2).

Analysis of the LDL cholesterol data (Table 4.2) revealed no significant effects for time, group or group*time (Table A4.2) over the intervention period. Additionally, following analysis of between-subject and within-subject effects the data for HDL cholesterol revealed no significant main effects for time, group or group*time (Table A4.2).

4.3.5 Nutritional intake

There were no significant interactions or main effects for time or group*time observed in total energy intake (Fig. 4.11), carbohydrate intake (Fig. 4.12), fat intake (Fig. 4.13) or protein intake (Fig. 4.14) during the 16-week intervention period in any group (SIT=6, IF=6, IFSIT=6). Analysis of energy intake on fasting days (Fig 4.15) detected no significant main effects of time or group*time, with no significant interaction effects between the two fasting groups. Nutritional intake on fasting days remained relatively constant over the course of the study, typically ranging between 500 kcal and 600 kcal.

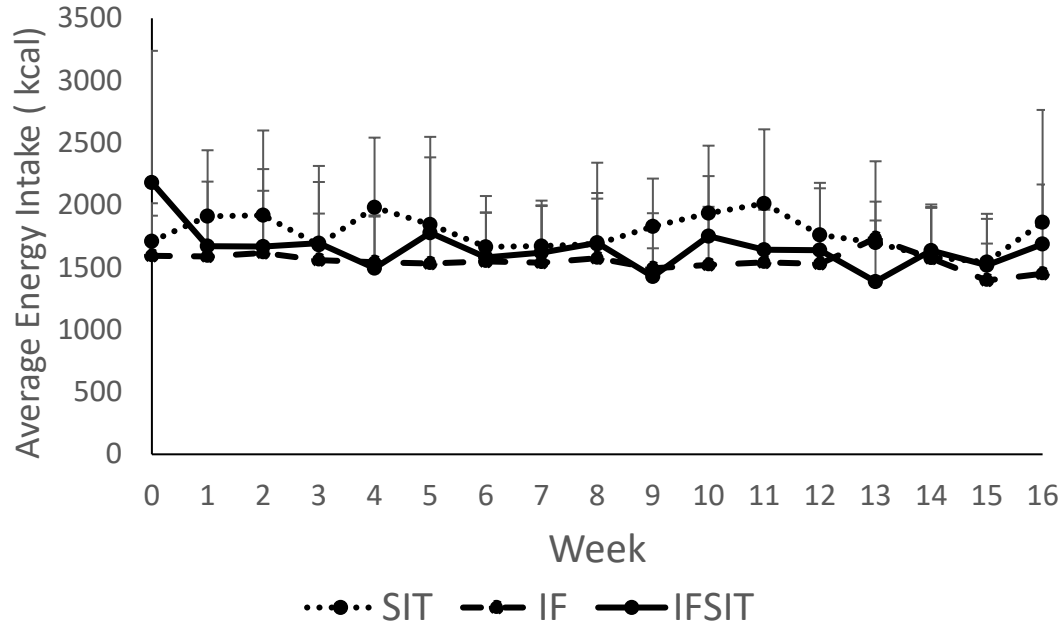


Figure 4.11 – *Changes in weekly caloric intake from baseline over a 16-week intervention period. Error bars represent ± S.D.*

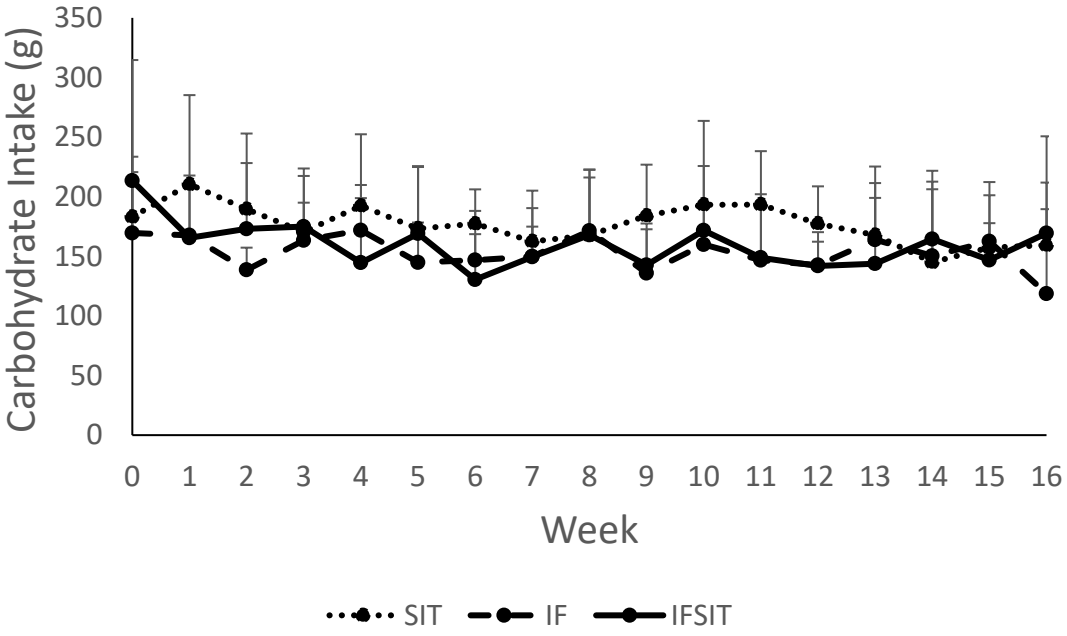


Figure 4.12 – *Changes in weekly carbohydrate intake from baseline over a 16-week intervention period. Error bars represent ± S.D.*

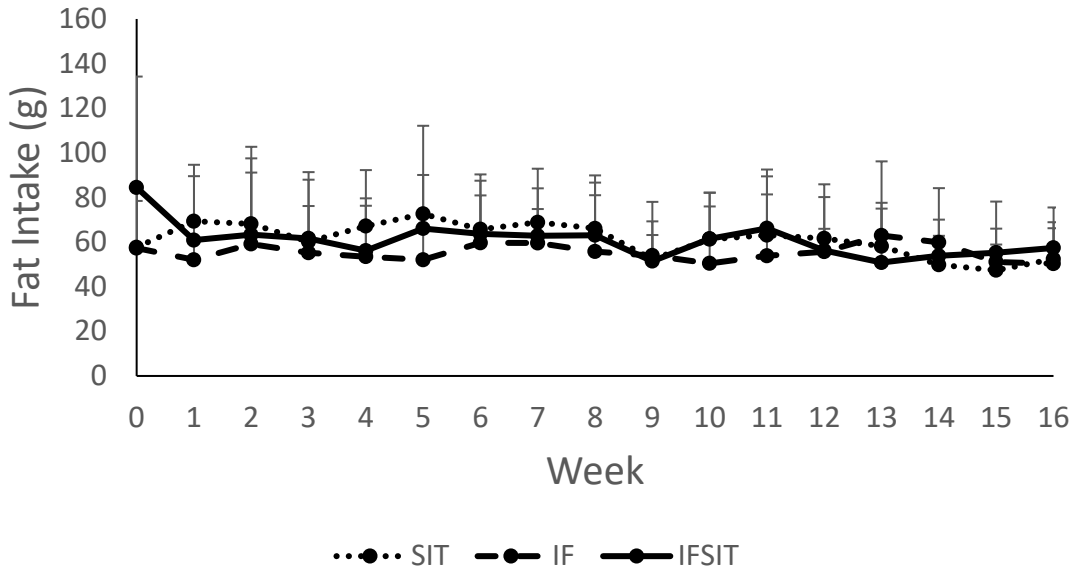


Figure 4.13 – *Changes in weekly fat intake from baseline over a 16-week intervention period. Error bars represent ± S.D.*

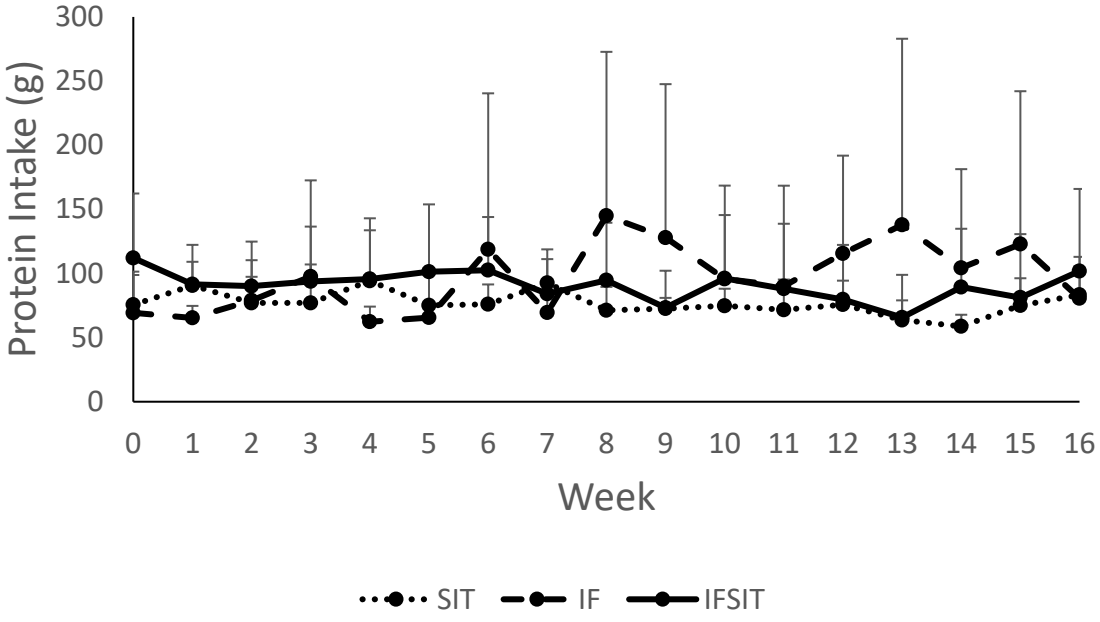


Figure 4.14 – *Changes in weekly protein intake from baseline over a 16-week intervention period. Error bars represent ± S.D.*

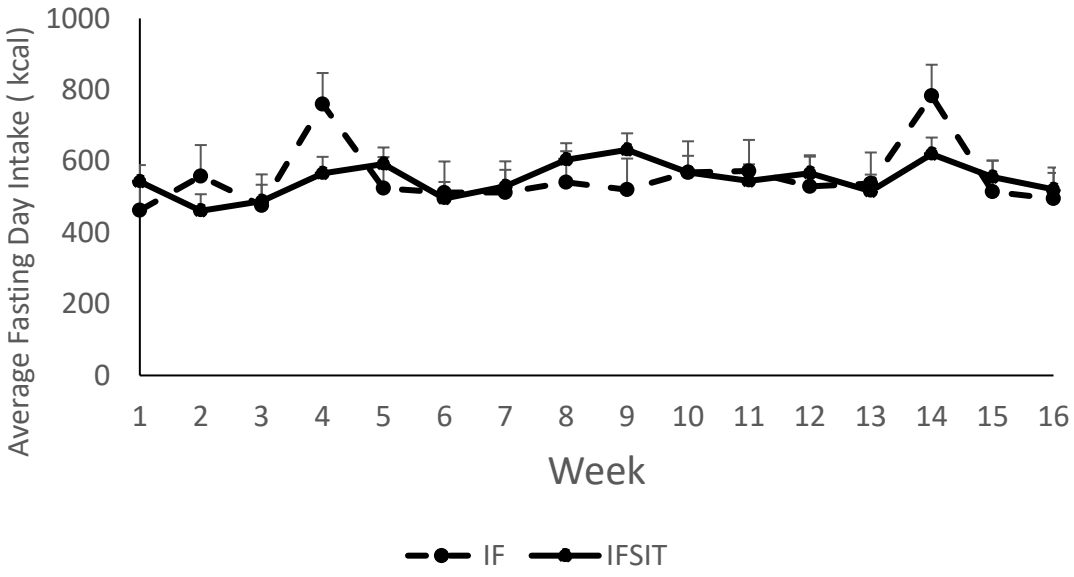


Figure 4.15 – *Changes in weekly energy intake from baseline on fasting days over a 16-week intervention period. Error bars represent ± S.D.*

4.4 Discussion

This study appears to confirm the hypothesis that IF, both alone and in combination with SIT, is effective for weight loss and for reduction in fat mass and BMI when compared to SIT alone. This study also found that while SIT was beneficial for enhancing aerobic fitness and retention of lean mass that it had no significant effect on factors relating to body weight and adiposity, suggesting that the hypothesised synergism between the protocols did not occur. Additionally, this study found evidence of enhanced LMM loss in the IFSIT group when compared to the IF and SIT groups alone that may limit the utility of combining IF and SIT in at-risk populations.

4.4.1 Anthropometric measurements and body composition

This study showed that SIT had little impact on body composition whilst IF reduced body mass, BMI, fat mass and lean mass and provides supporting evidence for the efficacy of IF as a viable weight loss strategy. Additionally, this study found that whilst SIT alone had no effect on weight and fat loss that it was able to maintain lean muscle mass when compared to non-significant reductions seen in the IF and IFSIT groups. Furthermore, sprint interval training increased aerobic capacity irrespective of the dietary status of the groups.

There were significant main effects observed for the 5:2 diet on body mass (Fig. 4.2), fat mass (Fig. 4.4) and BMI (Fig. 4.3) after 16 weeks of either IFSIT or intermittent fasting alone when compared to SIT. This provides strong support for the efficacy of the 5:2 diet on weight loss and the promotion of beneficial changes to body composition under free-living conditions. The loss of fat and body mass achieved was slightly less pronounced over 16 weeks (5% IFSIT and 5.8% IF), but still similar to other fasting studies using more stringent protocols (ADF, *m*ADF and fasting on 2 consecutive days per week), considering these studies are typically over 3 month weight loss periods (mean ≈ 6%) (Ash *et al.*, 2003; Varady *et al.*, 2011; Harvie *et al.*, 2013; Varady *et al.*, 2013; Trepanowski *et al.*, 2017). These results are also similar to studies using continuous energy restriction (mean ≈ 4.5%) (Varady *et al.*, 2011; Harvie *et al.*, 2013; Headland *et al.*, 2016; Schübel *et al.*, 2018). Interestingly, these results demonstrate smaller reductions than those reported by Schübel *et al.* (2018) who also used the 5:2 diet as the basis for their recent 12-week study and reported a 7.1% decrease in body weight.

Comparatively, there was little change in anthropometric measurements in the SIT group, most likely due to the absence of a specific dietary protocol provided to this group beyond adherence to nutritional guidelines and individual RDI (NHMRC, 2013).

The inability of SIT to reduce adiposity is noteworthy, though not entirely unexpected, given that there is some variability in the literature relating to the use of HIIT and SIT for weight loss, with relatively few studies reporting reductions in body weight due to the limited impact of these strategies on fat mass when used in the absence of energy restriction (Babraj *et al.* 2006; Trapp *et al.*, 2008; Ma *et al.*, 2013; Keating *et al.*, 2014).

In addition to the effect of intermittent fasting on reducing fat mass there was also a strong trend noted towards reductions in lean muscle mass (LMM), that appear to be slightly exacerbated by SIT. There was a near significant effect between the IFSIT and SIT groups (p=0.051) (Fig. 4.6) with decreases in LMM recorded in both fasting groups (IF= 1.1 kg and IFSIT= 1.8 kg) in comparison to a small increase in the SIT only group (0.43 kg). These results are comparable to other studies using 2 consecutive days of fasting per week where participants lost between 1.1 – 2.2 kg of lean mass over three-month intervention periods (Harvie *et al.*, 2011; Harvie *et al.*, 2013). These results suggest that the exercise protocol used was insufficient to offset the loss of muscle mass associated with fasting and appears to have increased muscle catabolism with 18.5% of total weight loss from lean mass in the IF group vs. 39.5% in the IFSIT group. This is noteworthy as typical lean mass loss during CR is between 14-23% (Beavers *et al.*, 2011) indicating that IF alone produces similar lean mass loss to CER but that IFSIT produces considerably more LMM loss in comparison. Examining the breakdown of lean body mass based on location (Fig. 4.7) shows there are reductions to lean mass in all body regions in both the IF and IFSIT groups, with the reductions in the IFSIT group being of greater magnitude, with no apparent attenuation of this effect in the legs despite undertaking 16 weeks of cycling training. In comparison, the SIT only group showed increases in all body regions excluding the trunk (2.7% decrease). Taken together these results indicate that SIT appears to exacerbate the loss of lean mass associated with IF. Whether this increased muscle catabolism is due to the supramaximal intensity of the exercise, compromised muscle synthesis caused by

reduced energy intake and available energy for protein synthesis or separate from negative energy balance entirely is unknown and will require further research to determine the underlying mechanisms.

Visceral fat levels (Table 4.2) decreased, albeit, non-significantly in both fasting groups while increasing slightly in the SIT only group. The results for the IF group are interesting as this group experienced its largest decline by week 8 (12.3%) decreasing by only a further 0.2% by week 16, possibly due to a decline in dietary compliance on fasting days. As expected, these declines in VAT corresponded to decreases in waist circumference, though this effect was are more apparent in the IFSIT group (average of 9 cm reduction) than the IF only group (average of 3 cm reduction).

The results for intramuscular fat content (Table 4.2) revealed a trend towards increasing levels at the 8-week testing period and then decreasing again by 16 weeks. This was measured in a relatively low number of participants compared to the total number of participants in each group (*n*=10 SIT, *n*=7 IF, *n*=5 IFSIT) and is a confounding factor in this analysis. Additionally, the use of the forearm as the area of interest may also be a factor given that the exercise was performed on a bicycle ergometer and the regional effects of exercise would not be captured using this target area. However, it is clear from the literature that global reductions in IMAT from both exercise and IER would be expected as VAT and IMAT are typically linked to total body adiposity (Janssen *et al.*, 2002; Boutcher, 2010). Given that many of the scans and analyses were performed by, or under the direct supervision of, an experienced radiographer the potential for experimental error is minimal.

4.4.2 Cardiovascular risk factors

4.4.2.1 Peripheral Blood Pressure

The peripheral blood pressure results (Table 4.2) obtained in the current study show that all groups experienced modest reductions in peripheral systolic blood pressure over the 16-week intervention period. Overall, these reductions amounted to 3.9% in the SIT group, a 3.2% reduction in the IF group and a 3.1% reduction in the IFSIT group. These data are presented after removal of outliers in the data set (>2 SD difference from mean).

The reductions obtained here would seem to indicate that all three interventions are effective at reducing blood pressure (Neter *et al.*, 2003). This trend was also apparent in the results obtained for peripheral diastolic pressure with a 3.7% reduction in the SIT group, a 2.6% reduction in the IF group and a 1.7% reduction in the IFSIT group following the removal of outliers.

The correlations between elevated blood pressure and cardiovascular risk and the associations of obesity and elevated blood pressure are both well understood (Ying *et al.*, 2015). The trends observed here, while largely non-significant, provide some support for previous findings of modest reductions blood pressure in at risk populations (Harvie *et al.*, 2011; Harvie *et al.*, 2013; Varady *et al.*, 2013; Ying *et al.*, 2015).

4.4.2.2 Central Blood Pressure

The results of central blood pressure (CBP) (Table 4.2) demonstrated a similar trend to the results for peripheral blood pressure with all groups producing small but non-significant reductions in central blood pressure with a significant effect of time detected in the data for systolic blood pressure only. The reductions amounted to a 2.3% reduction in systolic blood pressure in the SIT only group, a 3% decrease in the IF group and a 2.4% decrease in the IFSIT group. These results were also impacted by the presence of a small number of outliers (>2 SD difference from mean) within the data set that skewed the results obtained, likely due to acute lifestyle factors such as

diet, sleep status and stress levels. While diet wasn't controlled prior to testing (besides not being fasted) it is possible that unusual salt intake or some other dietary aberration may have influenced these outliers, the self-reported food diaries used were sufficiently sensitive to detect such changes. The results are presented here with these outliers removed.

The changes in diastolic pressure were similar, with a 3.6% decrease in the SIT group, a 3.6% decrease in the IF group and a 2.8% decrease in the IFSIT group. These results, while not significant, indicate that all intervention groups experienced decreases in CBP measurements. This is important given that there is a growing body of literature indicating that CBP (in combination with arterial compliance measures) might be more relevant when assessing cardiovascular risk and the prescription of antihypertensive therapies than peripheral blood pressure alone (Roman *et al.*, 2007; Kolade *et al.*, 2012; Supiano *et al.*, 2018). These small changes in both CBP and PBP are not unexpected given that most participants were normotensive at the beginning of the study, making it less likely that large changes in these values would be observed.

4.4.2.3 Heart Rate and pulse wave velocity

There were non-significant decreases in HR detected in all groups (Table 4.2), with this effect more being pronounced in both exercise groups, most likely as a function of increased cardiovascular fitness. Heart rate is an important biomarker of cardiovascular health, as reductions in heart rate have been demonstrated to be beneficial in cardiovascular disease progression and patient survival time (Böhm *et al.*, 2015). Conversely there was a small decline in heart rate in the IF only group at the week 8 mark, which had then increased again by week 16. To date, decreased heart rate has not been reported as an effect of either CR or IF in humans but has been reported in rats maintained on both CR and ADF diets (Mager *et al.*, 2006). In their

study Mager *et al.* (2006) demonstrated reductions to several parameters including HR, diastolic blood pressure, systolic blood pressure, body weight and increased vagal tone. However, the results obtained in the current study seem to provide some supporting trends (non-significant) of this effect in humans, though studies using a control group rather than comparison groups would be required to confirm this.

Pulse wave velocity is a clinical measure of arterial compliance that can be measured by either magnetic resonance imaging (MRI) or via the use of pressure waveform analysis performed using systems such as the SphygmoCor™ system. SphygmoCor™ uses tonometers in combination with pressure cuffs to provide measurements of the time delay between the carotid and the femoral pulses. Pulse wave velocity is predominantly used to predict cardiovascular risk in elderly and obese patients due to the correlation between both increased age and increased abdominal adiposity with reduced arterial compliance (Kolade *et al.*, 2012; Joly *et al.*, 2014). In the current study there was no significant change to PWV over the course of the 16-week intervention (Table 4.3), indicating that there was no influence of the protocols on arterial compliance within the relatively short timeframe of the study. It is worth noting however that most of the participants were well within the normal range for their respective age groups, providing less scope for change to these values.

4.4.2.4 Aerobic capacity testing

Aerobic fitness increased in both the SIT and IFSIT groups with improvements in VO_{2peak} recorded over the 16-week intervention period (Fig. 4.8). In contrast there was no difference observed in VO_{2peak} for the IF only group with values decreasing in all absolute and relative measures. One feature of these results is that the SIT group recorded lower values overall when compared to the IFSIT group in both relative and absolute VO_2 (Fig. 4.9). Whether these apparent differences are due to individual

variation or an underlying metabolic synergy between the 5:2 diet and SIT would require further study to determine.

Absolute VO_{2peak} results correlated well with the relative VO_{2peak} results with the exception of the IFSIT group whose absolute VO_2 scores were relatively unchanged between 8 and 16 weeks indicating that the observed changes in VO_{2peak} were likely influenced by weight loss rather than an increase in aerobic capacity given that absolute VO_2 is not influenced by changes in body mass. However, when changes in VO_2 are analysed in conjunction with changes in lean mass (Figure 4.10) the IFSIT group still had the largest increase in VO_{2peak} indicating that while muscle mass was decreasing there was still an apparent training effect in this group. Whether these changes are the result of some element of muscle fibre type switching, improved oxidative capacity or other alterations to other biochemical pathways relating to energy generation is unclear. A possible future research topic could be the use of muscle biopsies and fibre type analysis to investigate the underlying physiological and molecular changes impacted by fasting. Interestingly, the changes in VO_{2peak} were more pronounced in the IFSIT group when compared to the SIT only group irrespective of the VO_2 analysis used.

4.4.2.5 Plasma Lipid Measurements

There were no apparent changes in total blood cholesterol or triglycerides levels in any group, with only very small variations being recorded (Table 4.2). Similarly, there were small but non-significant changes in HDL and LDL levels with small decreases being recorded in all groups for LDL levels and slight increases in HDL levels recorded in both IF and SIT groups. Additionally, there was a small decrease in HDL in the IFSIT group (Table 4.2). To date the reported effects of IF on blood lipid levels have been largely positive, with most studies reporting decreases in triglycerides, LDL and total

cholesterol as well as some studies reporting increases to HDL levels within overweight or obese populations who were free of cardiovascular or metabolic disease. However, it should be noted that all of these studies used more stringent fasting protocols than the 5:2 diet (Harvie *et al.*, 2011; Kroeger *et al.*, 2012; Eshghinia & Mohammadzadeh, 2013; Harvie *et al.*, 2013; Varady *et al.*, 2013; Varady *et al.*, 2015). It is likely that the self-selection of foods on non-fasting days and the absence of changes to other lifestyle factors is the reason for the lack of change in lipid profiles. However, the limitations of the self-reported food intake used in this study make it difficult to confirm if this was indeed an influencing factor.

4.4.3 *Nutritional intake*

Self-reported average weekly caloric intake decreased slightly over course of the study period for the IF group (~5%) (Fig. 4.11), with noticeably decreased intake in the IFSIT group over 16 weeks (~14%) and a trend towards increased energy intake in the SIT (~5.6%) only group in most weeks, however, no significant main effects or interactions were detected. This may partially contribute to the lack of weight loss seen in the SIT group. Carbohydrate intake (Fig. 4.12) over the course of the study appeared to decrease slightly over the course of the study in all groups (SIT≈9.6%, IF≈6.6%, IFSIT≈15.9%) with no significant main effects or interactions detected. Fat intake was relatively unchanged in the IF only group (Fig. 4.13) with non-significant but apparent decreases in the IFSIT group (≈11.8%) and small increases in the SIT group (≈9.6%) over the 16-week intervention period. Protein intake remained relatively constant within the SIT and IFSIT groups with spikes of protein intake seen within the IF only group (≈55%) at weeks 7, 9, 10, & 14 (Fig. 4.14) with a general trend towards increasing protein intake towards the end of the study. Unfortunately, the reason why some participants consumed increased protein in those weeks is unclear due to the staggered recruitment used in this study. Energy intake on fasting days was consistent, though slightly higher than prescribed energy limits, with the weekly

average usually sitting between 500 & 600 kcal (Fig. 4.15) over the 16-week intervention period. Additionally, there were large increases in intake on fasting days during weeks 4 & 14, thought the reason for this is unclear.

The results obtained in this study correlate well with other studies reporting dietary and macronutrient intake with a number of HIIT/SIT studies reporting no significant changes to total energy or macronutrient intake (Janssen *et al.*, 2002; Trapp *et al.*, 2008; Corte de Araujo *et al.*, 2012; Keating *et al.*, 2014; Hamish & Sabo, 2016). Similarly, two IF studies that monitored dietary intake reported no significant changes to either total energy intake or macronutrient intake (Kroeger *et al.*, 2012; Trepanowski *et al.*, 2017), however another two studies did report decreases in average weekly energy intake and small decreases in macronutrient intake, with Harvie *et al.* (2011) reporting a decreased energy intake in both their IER and CER groups (IER=29.7, CER=20.5%) and Harvie *et al.* (2013) reporting 32% & 23% decreases for their IER groups and a comparable decrease in the CER group. The comparison of the current study to most of literature surrounding IF is complicated by the fact that most studies supply either the meals on fasting days or all meals to control energy and macronutrient intake. Even so, it appears that even when self-selecting food vs. directed feeding that there is no appreciable difference in energy intake or macronutrient favourability.

4.4.4 Recruitment, baseline measures and demographic information

There were significant differences observed in VO_{2peak} between groups. These differences were evident despite randomisation and subsequent attempts to match baseline characteristics to reduce such differences between the intervention groups. This occurred due to both the sporadic recruitment patterns and a larger than expected dropout rate seen during this study. Additionally, these factors led to near significant differences between observed in age, body weight and waist measurements between

the intervention groups. Due to lower than expected recruitment rates in the early stages of the study it was decided that the use of comparison groups would be necessary to ensure that the number of participants in each group was sufficient to achieve statistical power. The reason for the sporadic and initially poor recruitment appears to be largely due to the influenza pandemic that occurred during the initial stages of the study in addition to recruitment beginning in winter.

In summary, both IF and IFSIT were able to produce significant reductions in body weight and BMI when compared to SIT alone. Additionally, IF was able to produce significant reductions in fat mass when compared to SIT only. Decreases in lean mass were observed for both fasting groups, with greater reductions noted in the IFSIT group, indicating a potential underlying synergistic effect on muscle catabolism between the two protocols used. Significant increases in VO_2 were observed in the IFSIT group compared to IF only, but not between IF and SIT groups or IFSIT and SIT groups. These findings indicate that both IF and IFSIT are effective strategies for the reduction of body weight and metabolic risk but did not produce any significant reductions in cardiovascular risk factors associated with obesity despite increases in VO_2 in both exercise groups. Additionally, this study provides evidence that the SIT protocol used here is unable to influence weight loss either alone or when used in combination with IF.

Chapter 5

The effects of 16 weeks of sprint interval training and/or the 5:2 diet on inflammation, oxidative stress and risk factors associated with metabolic disease

5.1 Introduction

An increasingly automated, energy efficient society combined with widely available, palatable, energy dense food sources has resulted in alterations to both energy intake and energy balance. Furthermore, decreasing rates of physical activity have increased obesity rates globally, leading to a concomitant increase in the incidence of obesity related comorbidities such as cardiovascular disease and type 2 diabetes (WHO, 2010, WHO, 2018). Of these comorbidities one of the most common is the occurrence of metabolic syndrome, defined by a cluster of conditions that represent an elevated risk of CVD, CAD and type 2 diabetes. Metabolic syndrome is diagnosed when patients exhibit central obesity plus two or more of the following symptoms: hypertension, impaired fasting glucose, elevated blood triglyceride levels and decreased levels of HDL (IDF, 2006). Given that the risk of premature death is elevated by 2 to 3 times in patients with metabolic syndrome, there exists a pressing need to arrest weight gain and improve metabolic health in affected populations (IDF, 2006).

Insulin resistance is a central factor in both metabolic syndrome and Type 2 diabetes and develops as a consequence of a chronic oversupply of dietary energy and the ectopic accumulation of lipids in hepatic, visceral and intramyocellular tissues (Samuel and Schulman, 2016). This accumulation, particularly of diacylglycerides, results in impaired insulin signalling in the liver, white adipose tissue (WAT) and skeletal muscle, reducing insulin mediated glucose uptake in the muscles, hepatic glycogen synthesis and increasing the release of fatty acids from WAT (Reaven, 1988; Samuel and

Schulman, 2016). Consequently, blood glucose levels and increase compensatory insulin production in β-islet cells resulting in impaired glucose tolerance, hyperinsulinaemia and hyperglycaemia (Reaven, 1988; Samuel and Schulman, 2016).

Obesity induces chronic low-grade inflammation due to increased infiltration of WAT by macrophages (Bastard *et al.*, 2006). These macrophages secrete TNF-α, IL-1β and IL-6 which impairs insulin sensitivity through several different pathways including enhanced lipolysis in adipocytes, reductions in GLUT-4 and insulin receptor substrate-1 expression as well as other disruptions to insulin signalling (Bastard *et al.*, 2006). These alterations in adipokine levels have powerful effects on the induction of both IR and metabolic syndrome due to their roles in energy homeostasis. These include adiponectin's role in glucose sensitisation and fatty acid oxidation and the role of leptin in the control of macrophage activation, satiety, energy expenditure and in decreasing both plasma insulin and cortisol levels (Sørensen *et al.*, 1996; Bastard *et al.*, 2006; Chen *et al.*, 2015).

Treatment of obesity and metabolic syndrome is typically achieved using moderate energy restriction combined with increased physical activity. These approaches are non-pharmaceutical interventions that directly address the problem of persistent positive energy balance and facilitate weight loss. The associated reductions in central obesity and visceral fat lead to amelioration of both metabolic syndrome and insulin resistance (Bastard *et al.*, 2006). While successfully implemented in highly controlled and monitored studies the efficacy of these strategies has not been demonstrated in free–living populations.

In recent years SIT/HIIT and intermittent fasting have been proposed as replacements for MICT and CR as they are easier to follow, more time efficient and have similar effects on adiposity, glucose tolerance and insulin resistance (Harvie *et al.*, 2013; Gillen *et al.*, 2014; Carter *et al.*, 2016; Trepanowski *et al.*, 2017). This study aims to

test the hypothesis that the 5:2 diet and SIT both alone, and in combination will provide clinically significant improvements in inflammation, oxidative stress and other metabolic factors and that the combination. Additionally, this study will test the hypothesis that the combination of SIT and IF will provide an additive benefit. Thus, the aim of this study is to investigate the effects of the 5:2 intermittent fasting diet and sprint interval training (both alone and in combination) on risk factors associated with CVD, Metabolic syndrome, Type 2 diabetes and insulin resistance in a free-living population.

5.2 Materials and Methods

5.2.1 Oral Glucose Tolerance Testing

Participants were asked to fast overnight. Upon arrival at the laboratory a short peripheral catheter was inserted as per the procedure outlined in Chapter 3. Following consumption of a drink containing 75g of glucose (Gluco Scan 75, Sterihealth, Australia) blood samples were collected at baseline, 30 min, 60 min and 120 min. Where cannulation wasn't possible blood was collected by finger prick and analysed as below. Glucose tolerance results were reported as area under the curve (AUC) values using the trapezoidal method, with insulin resistance determined by homeostatic model assessment of insulin resistance 2 (HOMA2-IR) determined using the Oxford University calculator (https://www.dtu.ox.ac.uk/homacalculator/).

5.2.2 Blood collection and Analysis

Blood collection was performed as per chapter 3. Analysis of HBA1c and fasting glucose were carried out commercially (Dorevitch Laboratories, Australia). Serum insulin, ghrelin, C-reactive protein, IGF-1, TNF-α, leptin and IL-6 concentrations were determined in duplicate using commercial sandwich ELISA (Abcam, UK & Thermo Scientific, USA). All dilutions were performed using the recommended diluent buffer.

Sandwich ELISA and 8-Isoprostane analysis (competitive ELISA) where carried out as per the procedures outlined in chapter 3. Quality assurance data provided in Table 5.1 below.

Table 5.1 - Quality assurance data for competitive and sandwich ELISA assays

Test	Sensitivity	Interassay CV	Intraassay CV
C-reactive protein	10 pg/mL	9.7%	7.0%
8-soprostanes	1 pg/ml	1.8%	0.4%
Ghrelin	11.8 pg/mL	8.5%	6.0%
Leptin	3.5 pg/mL	4.6%	3.6%
Insulin	4 µlU/ml	12.0%	0.1%
Insulin like growth factor-1	0.1 ng/mL	12.0%	10.0%
Tumour necrosis factor-α	0.13 pg/mL	9.8%	8.5%

5.2.3 Visual analogue scale data

Attitudes to food and levels of satiety were assessed by self-reported visual analogue scale (VAS) questionnaires where responses were given by marking on a 100 mm scale to indicate their relative levels of pre- & post-meal hunger, fullness, desire for food, perceived capacity for food, palatability, mouth feel, smell, desire to consume the meal and Post meal satiety. A control meal was assigned to each participant to allow for consistent sampling in terms of timing and composition.

5.2.4 Statistical analysis

Statistical analysis was carried out as per Chapter 3.

5.3 Results

5.3.1 Glucose, glucose tolerance and insulin resistance

Table 5.2 details changes to plasma glucose over the intervention period in participants using the 5:2 diet, weekly sprint interval training or a combination of the two. Fasting glucose levels were relatively stable in all groups with only small variations recorded (± 0.1) Within-subjects, between-subjects and multiple comparisons analysis revealed no significant main or interaction effects identified (Table A5.1) for fasting glucose levels. Similarly, there was little variation (± 0.1) in HBA1c values over time in any group with no significant main or interaction effects (Table A5.1) detected (Table A5.1).

Table 5.2 – *Mean changes (± SD) in markers of glucose metabolism, glucose tolerance and insulin resistance relative to baseline levels after 8 and 16 weeks of intermittent fasting, sprint interval training or a combination the two.*

	SIT			IF			IFSIT		
	Week 0	Week 8	Week 16	Week 0	Week 8	Week 16	Week 0	Week 8	Week 16
		n=11			*n*=12			*n*=11	
Fasting Glucose (mmol/L)	4.9 ± 0.6	4.8 ± 0.7	4.8 ± 0.7	5.0 ± 0.6	4.8 ± 0.7	5.0 ± 0.6	4.9 ± 0.4	4.9 ± 0.3	4.8 ± 0.3
		n=10			*n*=12			*n*=11	
HBA1c (%)	5.1 ± 0.3	5.1 ± 0.4	5.1 ± 0.4	5.2 ± 0.3	5.1 ± 0.4	5.1 ± 0.4	4.9 ± 0.3	4.9 ± 0.3	5.0 ± 0.2
		n=11			*n*=12			*n*=11	
Glucose Tolerance (AUC)	697 ± 264	791 ± 159	822 ± 166	840 ± 223	808 ± 214	769 ± 192	817 ± 169	759 ± 137	714 ± 133
		n=9			*n*=7			*n*=8	
HOMA2-IR	1.6 ± 0.8	1.7 ± 0.6	1.8 ± 1.0	1.5 ± 0.5	1.3 ± 0.4	1.4 ± 0.5	1.7 ± 0.7	1.3 ± 0.6	1.5 ± 0.6

Analysis of glucose tolerance, measured as area under curve of glucose (Table 5.2), revealed no significant interaction or main effects (Table A5.1). Over the intervention period there was a small decrease in glucose tolerance in the SIT group, with small, comparable improvements in both fasting groups. Similarly, analysis of between-subjects within-subjects effects and multiple comparisons analysis (Table A5.1) revealed no significant main effects or interaction effects for HOMA2-IR (Table A5.1). HOMA2-IR values increased slightly in the SIT group but decreased in the fasting groups with the largest reduction in the IFSIT group (-0.2).

5.3.2 Metabolic hormone levels

Serum insulin levels (Table 5.3) decreased in all interventions with the largest decrease occurring in the SIT group (-16 µlU/ml), followed by the IFSIT group (-2 µlU/ml) and the IF group (-1 µlU/ml). Analysis of serum insulin levels by repeated measures ANOVA revealed no significant effects of time, group, group*time or interaction effects (Table A5.1).

Serum Ghrelin levels decreased in both the IF and SIT groups with the largest decrease occurring in the IF group, while the IFSIT group recorded increases at both time points. Analysis of between-subjects and within-subjects effects for IGF-1 levels (Table 5.3) showed no significant effects of time, group, group*time or interaction effects (Table A5.1).

Table 5.3 – *Mean changes (± SD) in metabolic hormone levels relative to baseline values after 8 & 16 weeks of intermittent fasting, sprint interval training or a combination the two.*

	SIT			IF			IFSIT		
	Week 0	Week 8	Week 16	Week 0	Week 8	Week 16	Week 0	Week 8	Week 16
		n=9			*n*=7			*n*=8	
Insulin (µlU/ml)	30 ± 5	20 ± 7	14 ± 8	12 ± 4	11 ± 4	11 ± 4	14 ± 6	10 ± 5	12 ± 5
		n=9			*n*=7			*n*=8	
IGF-1 (ng/ml)	32 ± 11	135 ± 6	86 ± 7	25 ± 29	30 ± 23	41 ± 37	58 ± 18	46 ± 18	38 ± 27
		n=9			*n*=7			*n*=8	
Ghrelin (pg/ml)	2690 ± 2178	2732 ± 1580	2122 ± 1484	4303 ± 2588	4586 ± 2792	3838 ± 2529	1605 ± 683	1808 ± 965	2296 ± 1570

Figure 5.1 details changes to serum leptin levels over the intervention period with equivalent decreases in both fasting groups in comparison to an increase in the SIT group. Analysis of between-subjects and within-subjects effects revealing significant time (p=0.014) and group*time (p=0.043) effects significant and a strong trend towards

an effect of group (p=0.065). Additionally, multiple comparisons analysis revealed a strong difference between the SIT and IFSIT groups (p=0.061).

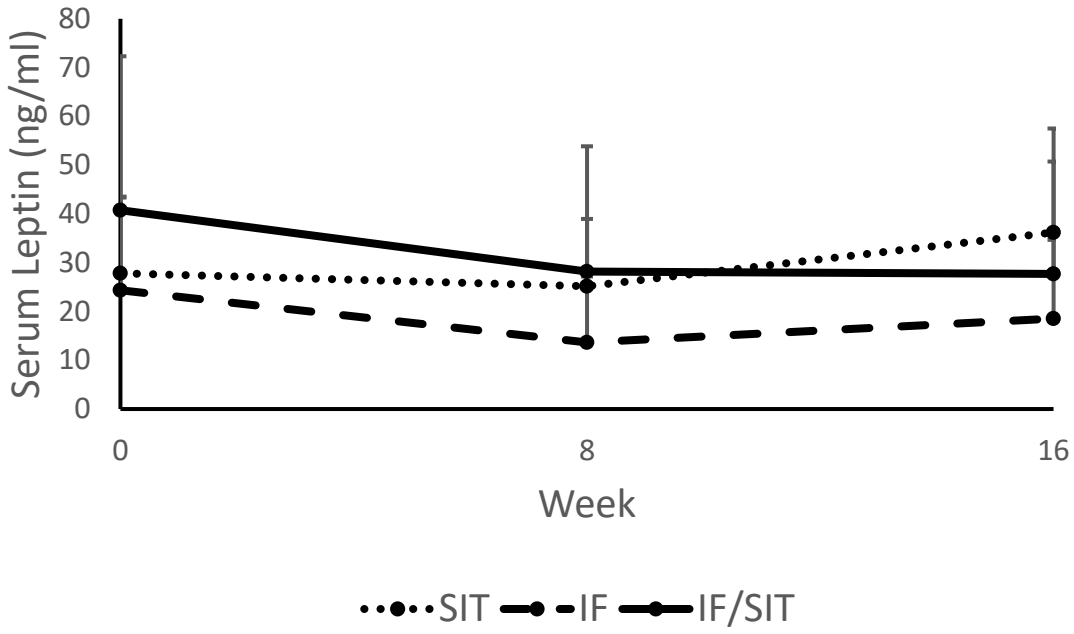


Figure 5.1 – *Mean changes in leptin levels over a 16-week intervention period. Data points represent week 0, 8 & 16. Error bars represent ± S.D.*

In contrast, there were no significant main effects for time, group or interaction effects reported for serum ghrelin levels (Table A5.1) over the 16-week intervention period. There was a however a significant group*time difference (p=0.046) (Table A5.1).

5.3.3 Inflammation & oxidative stress

Table 5.4 contains the data obtained for changes in C-reactive protein, TNF-α and isoprostane levels over the 16-week intervention period.

Table 5.4 – *Mean changes (± SD) in markers of inflammation and oxidative stress relative to baseline at 8 and 16 weeks of intermittent fasting, sprint interval training or a combination of the two.*

	SIT			IF			IFSIT		
	Week 0	Week 8	Week 16	Week 0	Week 8	Week 16	Week 0	Week 8	Week 16
		n=6			*n*=6			*n*=6	
TNF-α (pg/ml)	37 ± 47	14 ± 26	36 ± 49	99 ± 16	100 ± 16	94 ± 18	12 ± 26	4 ± 6	2 ± 5
		n=9			*n*=7			*n*=8	
C-reactive protein (ng/ml)	3429 ± 2579	2180 ± 1390	2981 ± 2337	2443 ± 2266	1985 ± 3463	1777 ± 2442	4253 ± 2914	3488 ± 3387	3727 ± 2559
		n=9			*n=7*			*n*=8	
Isoprostanes (ng/ml)	1896 ± 1060	1558 ± 569	1142 ± 180	1747 ± 522	1442 ± 324	1187 ± 345	1631 ± 455	1303 ± 512	1405 ± 375

TNF-α levels remained relatively steady during the study, with small decreases recorded in both fasting groups. Analysis of the data obtained for TNF-α levels (Table 5.4) identified no significant effects for time, group, group*time or interaction effects (Table A5.1). C-reactive protein levels were reduced in all groups, with comparable decreases in all groups. Additionally, there were no significant time, group, group*time or interaction effects (Table A5.1) noted in the results obtained for C-reactive protein levels (Table 5.4). Serum isoprostane levels decreased in all groups with the largest being recorded in the SIT group followed by the IF group and IFSIT groups. There were no were no significant effects for either group or group*time observed for serum isoprostane levels over the 16-week intervention period, there was however a significant effect for time (p=0.003) (Table A5.1).

5.3.4 Attitudes to Food and Satiety

Table 5.5 details the results obtained from visual analogue scale questionnaire analysis of participants attitudes and responses to food over a 16-week intervention period.

Participants experienced very little change in pre-meal hunger over the intervention period with no significant effects detected for time, group or group*time. Participant's

reported only small changes in desire for food, mouth feel and predicted capacity for food with no significant effects of time, group or group*time detected. There was, however, a significant effect for group*time detected in participant perceptions of taste (p=0.029), with no significant interactions detected between the groups in multiple comparisons analysis. Significant group (p=0.035) and group*time effects (p=0.044) were observed in sensations of smell (Table 5.5) with strong trends towards differences identified between the IF and SIT (p=0.053) and between the IFSIT and IF groups (p=0.088). Additionally, there were small increases in both post-meal desire for food and satiety in both the IF and IFSIT groups, however, these changes did not reach statistical significance.

Table 5.5 – *Mean visual analogue scale data relating to satiety, attitudes to food and desire to eat for participants completing 16 weeks SIT, IF or IFSIT.*

Parameter	Intervention	*n* =	Week 0	Week 4	Week 8	Week 12	Week 16
Hunger	SIT	9	5.4 ± 2.2	6.0 ± 3.9	6.1 ± 3.3	6.0 ± 3.2	5.1 ± 3.4
	IF	6	5.7 ± 1.5	7.5 ± 3.9	6.0 ± 3.2	5.0 ± 4.9	6.2 ± 4.1
	IFSIT	8	6.0 ± 1.1	6.2 ± 2.7	5.6 ± 4.3	5.9 ± 1.7	6.0 ± 1.7
Fullness	SIT	9	4.2 ± 2.6	4.2 ± 3.7	4.0 ± 4.2	4.5 ± 4.3	3.4 ± 3.8
	IF	6	5.0 ± 1.9	3.5 ± 4.9	5.6 ± 3.6	4.3 ± 4.6	6.6 ± 4.8
	IFSIT	8	4.4 ± 2.4	4.5 ± 1.8	4.6 ± 3.7	3.9 ± 2.1	6.1 ± 2.1
Desire to eat	SIT	9	5.0 ± 3.3	5.0 ± 2.6	5.7 ± 2.6	5.3 ± 2.8	3.6 ± 3.3
	IF	6	5.4 ± 2.8	6.8 ± 5.0	6.6 ± 1.9	5.8 ± 4.4	6.2 ± 3.9
	IFSIT	8	5.1 ± 2.6	5.5 ± 4.0	5.1 ± 3.6	5.4 ± 3.2	4.9 ± 3.6
Capacity for food	SIT	9	4.7 ± 2.7	5.1 ± 3.0	5.1 ± 2.5	6.6 ± 2.8	4.4 ± 2.7
	IF	6	6.2 ± 2.4	4.2 ± 3.0	5.6 ± 2.2	3.6 ± 2.7	5.2 ± 2.7
	IFSIT	8	5.4 ± 1.6	5.0 ± 2.6	5.7 ± 4.2	5.3 ± 2.2	5.4 ± 1.5
Taste	SIT	9	6.9 ± 1.6	6.8 ± 3.6	6.9 ± 3.5	7.4 ± 3.7	6.3 ± 3.6
	IF	6	6.6 ± 2.0	6.0 ± 4.8	7.8 ± 2.0	6.2 ± 3.7	7.4 ± 3.7
	IFSIT	8	7.4 ± 1.2	8.2 ± 1.0	7.3 ± 4.0	7.6 ± 0.7	7.9 ± 0.8
Mouth Feel	SIT	9	6.4 ± 2.1	6.4 ± 4.0	6.7 ± 3.4	6.9 ± 3.7	6.3 ± 3.2
	IF	6	6.5 ± 2.5	6.5 ± 5.7	7.4 ± 3.4	6. 7 ± 3.9	7.6 ± 3.6
	IFSIT	8	7.4 ± 1.8	7.5 ± 1.6	7.0 ± 3.6	6.9 ± 0.9	7.0 ± 0.8
Smell	SIT	9	7.3 ± 1.3	5.8 ± 3.4	6.1 ± 3.3	6.4 ± 3.5	6.1 ± 3.3
	IF	6	5.9 ± 2.7	5.7 ± 4.8	7.7 ± 2.1	6.8 ± 3.8	8.2 ± 4.1
	IFSIT	8	6.4 ± 1.7	7.4 ± 1.2	7.0 ± 4.4	6.9 ± 0.8	7.3 ± 1.3
Desire for food	SIT	9	6.6 ± 1.9	5.7 ± 4.1	5.2 ± 3.2	6.5 ± 4.1	5.4 ± 3.9
	IF	6	6.0 ± 3.2	6.3 ± 5.0	7.4 ± 2.2	6.5 ± 4.9	8.4 ± 5.6
	IFSIT	8	6.2 ± 1.2	6.5 ± 3.0	6.4 ± 4.6	6.1 ± 1.3	6.5 ± 0.7
Post-meal satiety	SIT	9	6.8 ± 2.0	7.6 ± 4.2	7.3 ± 4.1	6.8 ± 3.8	6.8 ± 3.8
	IF	6	7.1 ± 1.9	8.0 ± 3.6	9.0 ± 2.1	8.0 ± 3.1	8.6 ± 3.1
	IFSIT	8	7.9 ± 1.2	7.4 ± 2.3	7.7 ± 4.2	7.4 ± 1.1	8.1 ± 1.5

5.4 Discussion

In summary, there was little in the way of change in the metabolic markers examined in this study, with no significant changes to glucose metabolism, inflammatory markers and most metabolic hormones. This appears to disprove the initial hypotheses that there would be both significant improvements to these parameters using both IF and SIT and that the combination of the two protocols would provide additive benefit. There were however significant effects of time and group*time observed for leptin levels, a significant group*time effect observed in the ghrelin levels and a significant effect for time in isoprostane levels. A confounding factor in these analyses was that there were

some technical difficulties obtaining blood samples from some participants and poor antigen recovery in some ELISAs.

5.4.1 Serum glucose, glucose tolerance and HOMA2-IR

In the current study there was little change in metabolic parameters over the 16-week intervention period with no apparent change in HBA1c and HOMA2-IR in any group and only small increases in glucose tolerance (Table 5.2) in the IF and IFSIT groups. One factor that may have contributed is that most participants had relatively normal glucose tolerance, HBA1c and fasting glucose levels at baseline, meaning that large scale changes may have been less likely. Another confounding factor is the potential for dietary non-compliance as food intake was self-selected.

Closer examination of the raw data relating to glucose tolerance results for the IF group reveals that they were skewed slightly by two participants who had large decreases (>2 SD from the mean) in glucose tolerance, and that following removal of data from these participants from the analysis, the results for the two fasting groups are comparable (10.7% for IF & 11.1% for IFSIT). Similarly, to the IF group there were two participants within the SIT group that also experienced large decreases in glucose tolerance (>2 SD from the mean). When removed from the analysis there remains a slight trend towards increased glucose tolerance within this group, albeit a much smaller change than seen in the fasting groups at only 2%. While these results did not reach significance there were still modest improvements in the fasting groups, indicating a potential role for the 5:2 diet in the improvement of glucose metabolism in populations suffering obesity.

Changes in glucose tolerance appeared to be independent of any change in HOMA2-IR (Table 5.2), indicating that the interventions used were likely insufficient to reverse the insulin resistance present within the participant groups despite causing

improvements in body composition and small improvements in glucose metabolism. Self-guided food selection may be problematic and have influenced the outcomes. The limitations of self-reported food intake make it impossible to speculate on the role of dietary intake during this study. Additionally, there was a high degree of variation within the sample population that was likely a confounding factor in the analysis.

Glucose tolerance improved marginally, albeit not significantly, in comparison to the significant improvements to insulin resistance and glucose tolerance reported in other studies. These studies investigating HIIT and SIT protocols have reported significant improvements to both glucose tolerance and insulin resistance despite differences in both protocol and exercise intensity (Metcalfe *et al.*, 2012; Gillen *et al.*, 2014; Little *et al.*, 2014). Motivational factors relating to the performance of maximal sprints during the exercise intervals may have influenced these factors in the current study as several participants had trouble sprinting effectively despite repeated encouragement, resulting in participants struggling with the expected load requirement leading to laboured effort in the later stages of training.

There may be a sex-related difference in metabolic responses to SIT and HIIT protocols in relation to insulin resistance. Two studies have demonstrated that insulin sensitivity was increased in men but not in women following bouts of HIIT and SIT training over a 6-week period. Given the proportion of women (*n*=28) vs men (*n*=6, SIT=2, IF=1, IFSIT=4) in the current study it is likely that these differences affected the results, particularly as the outcomes of the current study exhibit the same trends observed in Metcalfe *et al.* (2012) and Gillen *et al.* (2014). Gillen *et al.* (2014) demonstrated that these differences were likely due to an approximately six-fold increase in muscle GLUT4 protein content in males after several weeks of training when compared to the changes seen in women, though the authors did note that small sample sizes may have led to potential type II errors. Metcalfe *et al.* (2012) also theorised that the sex related differences could be due to differences in the rate of

glycogen depletion between the sexes but, too date, this has yet to be tested in a controlled study.

5.4.2 Metabolic Hormones

The sample sizes for these data were smaller than those for the cardiovascular and anthropometric data as there was poor antigen recovery from some ELISA assays, additionally, blood samples were unable to be obtained from some participants (Table 5.3).

Over the course of the 16-week intervention period there were modest, non-significant decreases in serum insulin (Table 5.3) levels in all groups. At week 8 the change in insulin levels in the IFSIT group was greater than in the IF group (10% difference) but by week 16 the magnitude of the difference had decreased to 7%. These results show similar trends as the effects reported in the literature, where both IF and SIT have both been reported to significantly decrease insulin concentrations (Harvie *et al.*, 2011; Harvie *et al.*, 2013; Gillen *et al.*, 2014). The reasons for this are unclear due to the limitations of self-reported dietary intake and other factors affecting a free-living study population but could be due to the combined effect of SIT and IF on thermogenesis or other signals relating to appetite control.

Serum IGF-1 levels (Table 5.3) were relatively unchanged in the IF and IFSIT groups during the study with only small, non-significant increases recorded by week 16 (9% IF & 13% IFSIT). Comparatively there was a non-significant increase in the SIT group at both week 8 and 16 time points. In all groups there were no significant interaction effects detected, largely due to the variation within the data set. Despite this, these results correlate well with what has been reported in other studies, with two human studies reporting no change in IGF-1 levels following intermittent fasting (Harvie *et al.*, 2011; Harvie *et al.*, 2013) and another only reporting a significant change in

participants consuming a liquid based diet (Kroeger *et al.*, 2012). Additionally, SIT has been demonstrated to promote increased IGF-1 levels, which given its anabolic nature is not unexpected (Meckel *et al.*, 2011; Cui *et al.*, 2015).

There was a near significant decrease detected in serum leptin levels (Fig. 5.1) in the IFSIT when compared to the SIT group (p= 0.067). While leptin levels decreased in both fasting groups the effect appears to have been more profound at 8 weeks when compared to 16 weeks, with greater reductions at both time points observed in the IF group when compared to the IFSIT (IF: 54% & 22%, IFSIT: 42% & 17%). Comparatively, levels of leptin were higher at both time points in the SIT only group with an overall increase of 37%. These results correlate well with other IF studies that have also demonstrated decreases to leptin concentrations (Halberg *et al.*, 2005; Harvie *et al.*, 2011; Klempel *et al.*, 2012; Harvie *et al.*, 2013; Varady *et al.*, 2013), however those SIT studies that report leptin concentration generally report lower levels (Corte de Araujo *et al.*, 2012; Sevits *et al.*, 2013) in comparison to the increase seen here. It is worth noting that the influence of exercise on leptin concentrations shows more variability than fasting (Pérusse *et al.*, 1997).

Serum ghrelin levels (Table 5.3) showed a trend towards decreased levels in the IF (10.5%) and SIT (17.7%) groups over the 16-week intervention period, though these changes were not statistically significant. Comparatively, ghrelin levels increased in the IFSIT group at both time points which is unexpected given the results seen in the other groups, particularly as the IFSIT protocol is a combination of the other protocols. To date there has only been a small number of IF related studies that have reported ghrelin levels in humans, which were unchanged after both 6 months and 22 days of IF (Heilbronn *et al.*, 2005; Harvie *et al.*, 2011). Conversely, the results obtained in this study correlate well with several investigations that have reported reductions to ghrelin concentrations in participants undertaking several weeks of sprint interval training (Sim *et al.*, 2014; Metcalfe *et al.*, 2015; Holliday & Blannin, 2017).

The combination of decreased leptin levels and decreased ghrelin is interesting given their roles in satiety and hunger with the results indicating that there is likely reduced stimulation of hunger via ghrelin and decreased inhibition of hunger signals via leptin. While the reduced stimulation of hunger via ghrelin would be beneficial in terms of energy balance and the control of energy intake the reductions in leptin are likely tied to the amelioration of the hyperleptinaemia typically found in obesity rather than inhibition of hunger signals.

5.4.3 Inflammation and oxidative stress

The data for inflammatory and oxidative stress markers has smaller sample sizes than the anthropometric and cardiovascular data due to technical and analytical difficulties that influenced the statistical power (Table 5.4). TNF-α and C-reactive protein were measured to determine the effects of the protocols on inflammatory markers. Both IF and IFSIT groups experienced decreases in TNF-α levels (Table 5.4) in contrast to an overall increase at week 16 in the SIT only group. While not significant the trends observed in the current study support the trends observed previously in the literature, with many studies relating to the effects of IF on TNF-α reporting either small decreases to TNF-α levels or no change (Halberg *et al.*, 2005; Kroeger *et al.*, 2012; Harvie *et al.*, 2013). While, comparatively, those studies investigating the effects of HIIT & SIT have typically reported significant increases in TNF-α levels (Meckel *et al.*, 2011; Zwetsloot *et al.*, 2014; Hamish & Sabo, 2016).

There was a non-significant decrease in C-reactive protein levels observed in the IF group and non-significant increases in both the IFSIT and SIT groups (Table 5.4). A small number of fasting studies have reported significant reductions in blood CRP levels (Harvie *et al.*, 2011; Varady *et al.*, 2013), while others have reported either very small changes or no change at all (Kroeger *et al.*, 2012; Trepanowski *et al.*, 2017).

These variations are likely due to the differing fasting protocols and intervention periods used between studies, with Trepanowski *et al.* (2017) and Varady *et al.* (2013) using *m*ADF, Kroeger *et al.* (2012) using a single day of fasting per week and Harvie *et al.* (2011) using 2 consecutive days of fasting per week. Comparatively, those studies that have measured CRP response to SIT and HIIT have reported only small, non-significant changes, making the results in the current study slightly anomalous. The reasons for this, as with the fasting studies, could be related to differences in protocols, variation within the sample population or other factors such as the timing of blood collection and the aforementioned motivational factors relating to SIT.

Serum isoprostane levels (Table 5.4) showed a tendency for small but non-significant decline in all intervention groups over time but without significant difference between the groups. These results indicate that both IF and SIT might be effective for reducing oxidative stress in overweight/obese populations albeit not significantly. This correlates well with other studies that have concluded that IF is effective for the reduction of oxidative stress, however this finding is not a universal with several studies also reporting no change to markers of oxidative stress (Johnson *et al.*, 2007; Harvie *et al.*, 2011; Klempel *et al.*, 2012; Kroeger *et al.*, 2012; Harvie *et al.*, 2013).

5.4.4 Visual analogue scale analysis

There were significant group* time differences observed in participant VAS data (Table 5.5) relating to sensations of taste and smell and a significant effect of group for sensations of smell. But otherwise there were no significant main effects or interactions observed. Interestingly, the results obtained here did not seem to correlate with ghrelin or leptin levels as there were only small shifts in indices relating to hunger and satiety, with the exception of non-significant decreases to the desire to eat in the SIT and IFSIT groups (Table 5.5).

Overall while many of the observed changes were small and non-significant their presence indicates a general reduction in cellular metabolic stress with both fasting and exercise. The changes in oxidative stress markers in combination with the reductions in leptin and ghrelin levels would be of great value in terms of improving the general metabolic health of obese and overweight populations, particularly in terms of reducing both the hyperleptinaemia and chronic inflammation seen in obesity.

Chapter 6

The effects of 16 weeks of sprint interval training and/or the 5:2 diet on psychological parameters associated with mood state, satiety and exercise and dietary compliance

6.1 Introduction

While the focus of obesity research is primarily geared towards treating the somatic effects of obesity there is large body of evidence supporting a strong association between obesity and its negative effects on mental health. These negative effects are particularly relevant to both major depressive disorder (MDD) and anxiety, as the chronic nature of these conditions increases with BMI (Petry *et al.*, 2008; Simon *et al.*, 2008; Opel *et al.*, 2015). However, to date, there has been little data defining causative links between the two conditions despite strong correlations.

Several studies indicate that the relationship between depression and obesity may be bidirectional with depression leading to several factors that predispose patients to obesity and obesity leading to several psychosocial and biological changes that predispose patients to MDD (Strine *et al.*, 2008; Simon *et al.*, 2008; Marmorstein *et al.*, 2014). Obesity, in addition to its health effects, is stigmatised within society and has the potential to damage self-esteem, diminish sexual function and to limit mobility, potentially contributing to the onset of depression (Wee *et al.*, 2015; Marmorstein *et al.*, 2014). Depression also leads to potential side effects that can predispose sufferers to weight gain due to the negative side effects associated with medications, changes to appetite/food intake, lethargy and alterations to sleep patterns (Fava *et al.*, 2000).

Overeating and oversleeping (increased energy intake and decreased energy expenditure) have been strongly correlated with MDD and appear to be common

methods used by sufferers to escape the effects of depression with periods of overeating typically preceded by an increase in depressive symptoms (Gianini *et al.*, 2013). This is especially troubling given the correlations between obesity and the incidence of depression, potentially leading to cyclic patterns of overeating and oversleeping that potentiate an increase in depressive symptoms. Thus, understanding and managing lifestyle-based protocols could relieve depressive symptoms and facilitate weight loss in these patients through alterations in energy balance.

There are additional complexities that further influence the association between obesity and depression, with some studies implicating both age and sex as contributing factors. An example of this occurs in adolescent girls where early onset of depression leads to an elevated risk of obesity in later adolescence, and that, this in turn, increases the risk of depression in early adulthood (Marmorstein *et al.*, 2014). Regardless, the strength of the association between obesity and depression is underscored by an observed decline in symptoms when patients lose weight in response to dietary advice and increased physical activity (Kloiber *et al.*, 2007).

There is also a large body of evidence (Craft *et al.*, 2004; Salerian *et al.*, 2008; Zschucke *et al.*, 2013; Goldberg *et al.*, 2014) connecting exercise with improvements to mood state, depression and several other psychological disorders. However, to date, the underlying mechanism for these changes is yet to be fully elucidated (Zschucke *et al.*, 2013). Mental health is multifaceted in both its causes and symptoms, but exercise has been demonstrated to induce beneficial changes to neurotransmitter levels, increase production of endorphins and alter brainstem responses to exercise related thermogenesis (Craft *et al.*, 2004; Salerian *et al.*, 2008; Goldberg *et al.*, 2014). While there is still some debate surrounding effective exercise protocols that may improve mental health outcomes there is a clear correlation between exercise,

particularly long-term exercise, and the amelioration of depressive symptoms (Zschucke *et al.*, 2013).

While long term CR and MICT have historically been used in the treatment of obesity, their success in mainstream treatment and prevention has been limited despite having beneficial effects on MDD, anxiety and several other conditions (Trepanowski *et al.*, 2011; Zschucke *et al.*, 2013). While MICT has previously been utilised due to its greater fat oxidation potential (Romijn *et al.*, 1993) it has been shown to suffer from poor compliance in longer-term programs. This is largely due to lifestyle factors such as participants having limited time and anecdotally reporting boredom and low motivation as reasons for non-compliance (Sallis *et al.*, 1990). Dietary non-compliance also limits the effectiveness of these protocols as there are several potential motivational, lifestyle, hormonal and metabolic hurdles that limit the success of dietary interventions, particularly in free-living populations. To improve patient compliance with exercise recommendations there has been growing support for the use of supramaximal (SIT) and maximal/submaximal (HIIT) interval training over MICT as a more effective protocol for the reduction of risk factors associated with cardiovascular and metabolic disease (Weston *et al.*, 2014; Milanović *et al.*, 2015; Ramos *et al.*, 2015).

To improve compliance with dietary recommendation an emerging alternative to CR is the practice of intermittent fasting, i.e. periods of severe energy restriction interspersed with periods of normal eating (Trepanowski *et al.*, 2011). Reductions in anger, depression, tension, fatigue, confusion and total mental disturbance scores as well as an improved vigour scores (assessed by profile of mood states questionnaire) have been reported using intermittent fasting (Harvie *et al.,* 2013), which is in line with the positive effects of IER reported during Sunnah fasting in Islam (Teng *et al.*, 2011; Hussin *et al.*, 2013). While encouraging there is a need for more specific studies focussing on these parameters to further understand the effects of IF on MDD.

However, while these effects have been well documented using CR, there has been little information regarding the effects of these protocols on mood state and depression. Those studies that have reported the effects of SIT and HIIT on mood state have generally reported positive effects in sedentary individuals at risk of metabolic disease. However, it appears that these improvements in mood state may be less pronounced in trained individuals (Freese *et al.*, 2014; Nalçakan *et al.*, 2014; Selmi *et al.*, 2018).

This study aims to determine the effects of the 5:2 intermittent fasting diet and sprint interval training on mood state and quality of life measures over a 16-week intervention period to test the hypothesis that 5:2 diet and SIT protocols will promote significant improvements to these factors. Additionally, it will test the hypothesis that there will be an additive benefit to using both protocols in combination. Additionally, this study aims to determine the effects of these protocols on satiety, dietary habits and changes to physical activity patterns.

6.2 Materials and Methods

6.2.1 Psychological and quality of life data

Psychological parameters and quality of life data were assessed by self-reported questionnaires. Profile of mood states (long form) (POMS), international physical activity questionnaire (self-administered, long form) (IPAQ) and Short Form 36 (SF-36) questionnaires were uploaded to Qualtrics and administered as required by emailing the appropriate links to participants. Profile of mood states was administered every 4 weeks, IPAQ was administered weekly and SF-36 was administered at both baseline and at 16 weeks. Sitting time and activity levels were assessed using the IPAQ questionnaire.

6.2.2 Exercise Performance and Compliance

Participants allocated to exercise groups attended three sessions per week and performed between 4-6 bouts of exercise at 150% of VO_{2peak} based on maximum load obtained during the aerobic capacity testing as outlined in Chapter 3. Upon arrival participants were fitted with a heart rate monitor (Polar V800, Polar, Finland) and asked to rest for 5 minutes to attain a resting heart rate. During the exercise period heart rates were also recorded at the conclusion of the third and final intervals. Participants who attended less than 42 of the 48 exercise sessions were excluded from the final analysis.

6.2.3 Statistical analysis

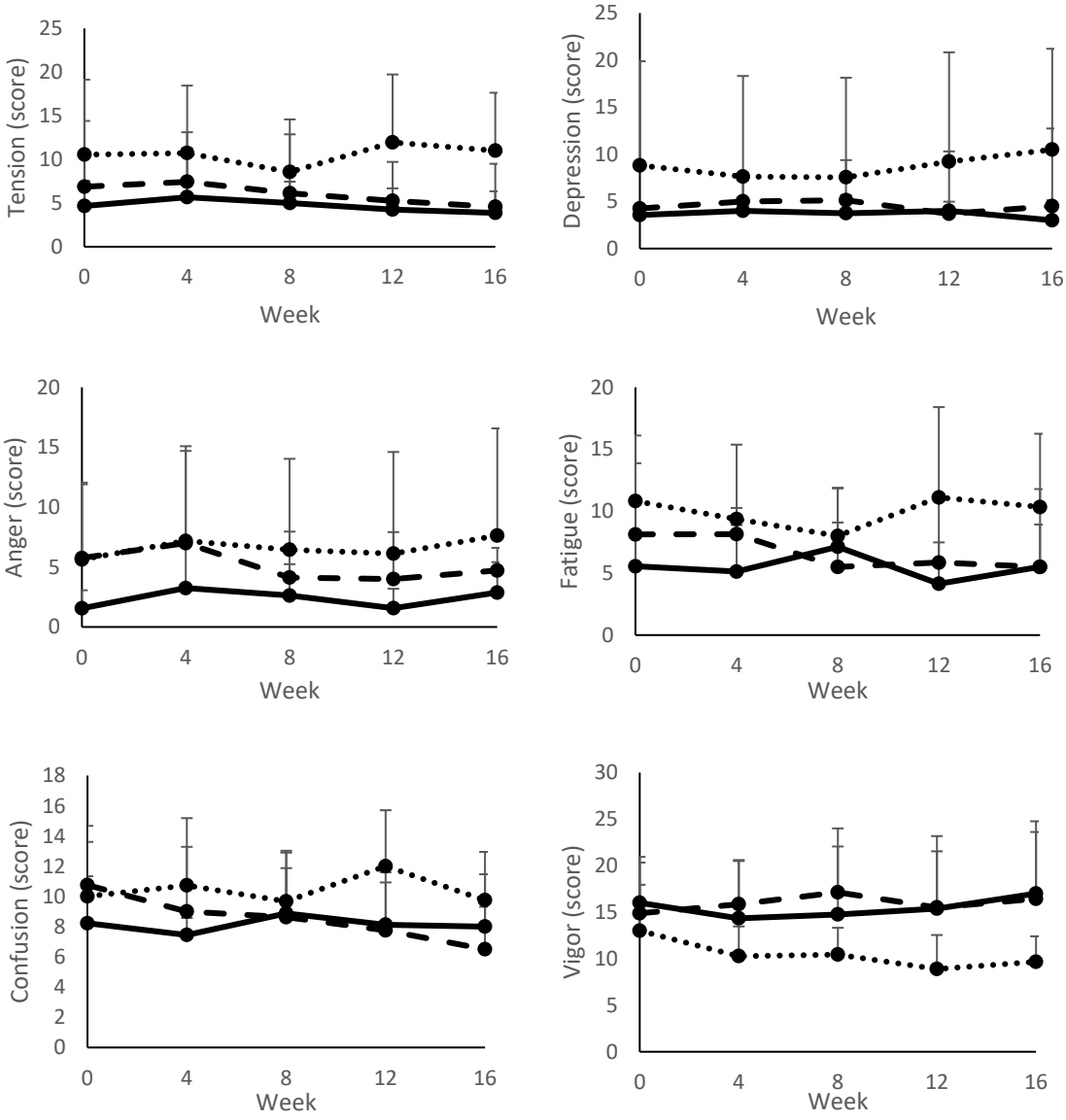
Statistical analysis was carried out as per Chapter 3.

6.3 Results

6.3.1 Psychological parameters and quality of life measures

Following analysis of the profile of mood states questionnaire (POMS) (SIT=9, IF=8, IFSIT=9) data by repeated measures ANOVA no significant main effects for group or group*time were observed for tension, depression anger or fatigue. Similarly, there were no significant main effects for group or group*time observed for confusion, vigour and total mental disturbance indices over the 16-week intervention period. There were, however, significant effects for time noted in both confusion (p=0.010) and total mental disturbance (p=0.033), but not in the other factors. Additionally, there were also no significant interactions between groups detected following multiple comparisons

analysis. Table A6.1 details the statistical findings of the between-subjects and within-subjects analysis for the profile of mood states.



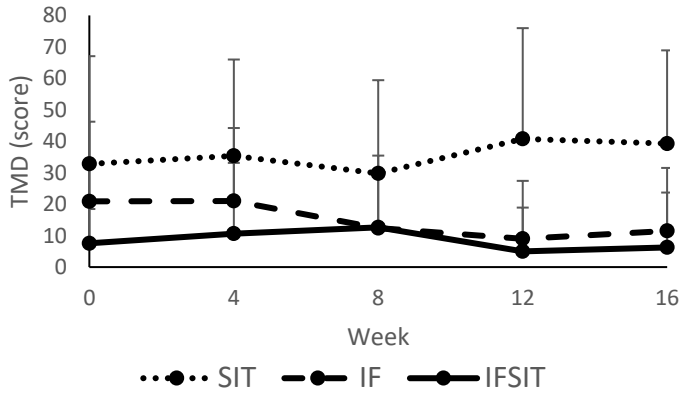


Figure 6.1 – *Change in mean profile of mood state questionnaire scores from baseline for participant groups undertaking 16 weeks of IF, IFSIT or SIT only. Results presented (L-R & top to bottom) for tension, depression, anger, fatigue, confusion, vigour and total mental disturbance. Error bars represent ± S.D.*

Table 6.1 details quality of life measurements obtained by SF-36 questionnaire for participants carrying out 16 weeks of IF, SIT or a combination of both protocols (SIT=7, IF=8, IFSIT=8). Following analysis no significant effects for time, group or group*time detected between values at baseline and at the end of the 16-week intervention period for indices relating to physical functioning (Time p=0.086; Group p=0.614; Group*Time p=0.840), physical limitations (Time p=0.350; Group p=0.966; Group*Time p=0.139), emotional limitations (Time p=0.634; Group p=0.379; Group*Time p=0.285), energy levels (Time p=0.098; Group*Time p=0.311), emotional well-being (Time p=0.946; Group p=0.216; Group*Time p=0.641), social functioning (Time p=0.869; Group p=0.189; Group*Time p=0.433), pain (Time p=0.435; Group p=0.137; Group*Time p=0.700) and general health (Time p=0.088; Group p=0.063; Group*Time p=0.465). There was however a significant effect for group noted for participant energy levels (p=0.017), with a significant improved energy levels in the IFSIT group compared to the SIT group (p=0.013) and a strong trend towards improved general health in the IFSIT group when compared to the SIT group (p=0.052).

Table 6.1 – *Mean short form 36 questionnaire* (*SF-36) data for participants completing 16 weeks SIT, IF or IFSIT.*

Parameter	Intervention	*n* =	Week 0	Week 16
Physical Functioning	SIT	7	87.27 ± 11.7	87.14 ± 11.1
	IF	8	85.63 ± 10.8	90.00 ± 10.5
	IFSIT	8	85.21 ± 9.9	92.50 ± 8.8
Physical Limitations	SIT	7	84.09 ± 23.1	96.43 ± 9.4
	IF	8	96.88 ± 8.8	81.25 ± 40.1
	IFSIT	8	90.63 ± 26.5	83.33 ± 30.3
Emotional Limitations	SIT	7	81.82 ± 31.1	85.71 ± 26.2
	IF	8	70.08 ± 44.1	88.89 ± 27.2
	IFSIT	8	87.50 ± 0.0	83.33 ± 27.9
Energy/Fatigue	SIT	7	40.45 ± 17.7	45.00 ± 15.3
	IF	8	51.25 ± 23.0	61.67 ± 23.2
	IFSIT	8	63.75 ± 16.6	67.50 ± 10.8
Emotional Well-being	SIT	7	68.73 ± 21.8	68.57 ± 20.0
	IF	8	76.00 ± 20.6	73.33 ± 16.5
	IFSIT	8	80.50 ± 18.6	84.67 ± 11.4
Social Functioning	SIT	7	78.18 ± 25.0	78.57 ± 18.7
	IF	8	82.81 ± 21.1	81.67 ± 12.9
	IFSIT	8	87.50 ± 25.9	89.58 ± 12.3
Pain	SIT	7	81.14 ± 13.9	81.79 ± 12.2
	IF	8	80.94 ± 15.0	91.25 ± 8.3
	IFSIT	8	83.75 ± 10.4	80.42 ± 11.1
General Health	SIT	7	54.52 ± 19.7	56.34 ± 19.7
	IF	8	63.13 ± 17.5	69.17 ± 13.6
	IFSIT	8	70.00 ± 14.6	78.33 ± 9.8

6.3.2 Sitting time and activity levels

The average weekly sitting time (Fig. 6.2) (SIT=10, IF=8, IFSIT=8) using repeated measures ANOVA there was found to be significant time (p=0.020) and group*time (p=0.006) (Table A6.2) effects, with a strong trend indicated between the IFSIT and SIT groups (p=0.059). In comparison there were no significant main effects for time, group or group*time detected (Table A6.2) for moderate activity (Fig. 6.3), though trends were identified for changes to group (p=0.099) and group*time (p=0.090). Analysis of walking activity during the intervention period revealed no significant effects of time or group*time but did reveal a trend towards a difference between groups (p=0.065), with a near significant difference between detected during multiple comparisons analysis (p=0.065) between the IF and SIT groups with an overall decrease in walking observed in the IF group over the course of the study. Additionally, there were no significant effects for group, group*time, time or interaction effects identified for vigorous activity (Fig. 6.4) or total activity (Fig. 6.6).

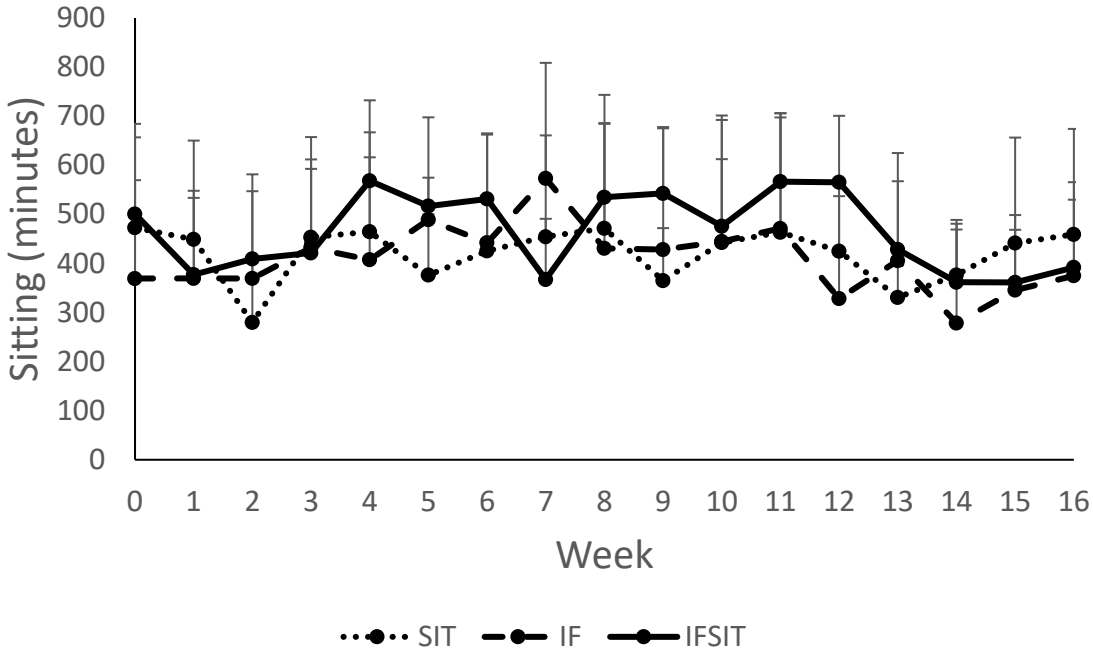


Figure 6.2 – *Mean changes to weekly sitting time from baseline over a 16-week intervention period in participants undertaking 16 weeks of IF, IFSIT and SIT. Error bars represent ± S.D.*

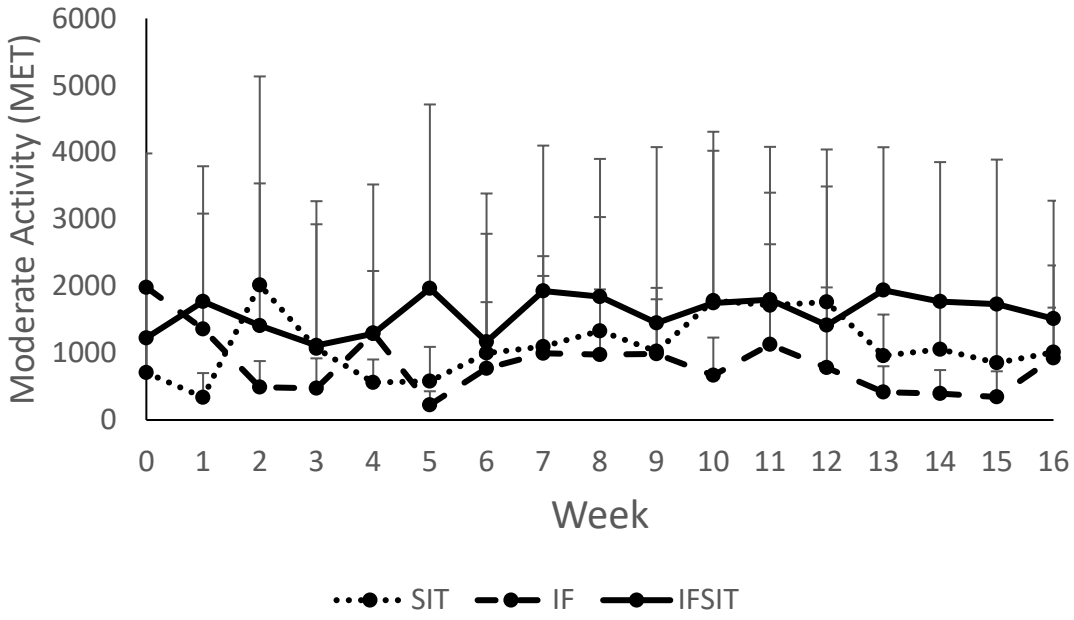


Figure 6.3 – *Mean changes to moderate activity from baseline over a 16-week intervention period in participants undertaking 16 weeks of IF, IFSIT and SIT. Error bars represent ± S.D.*

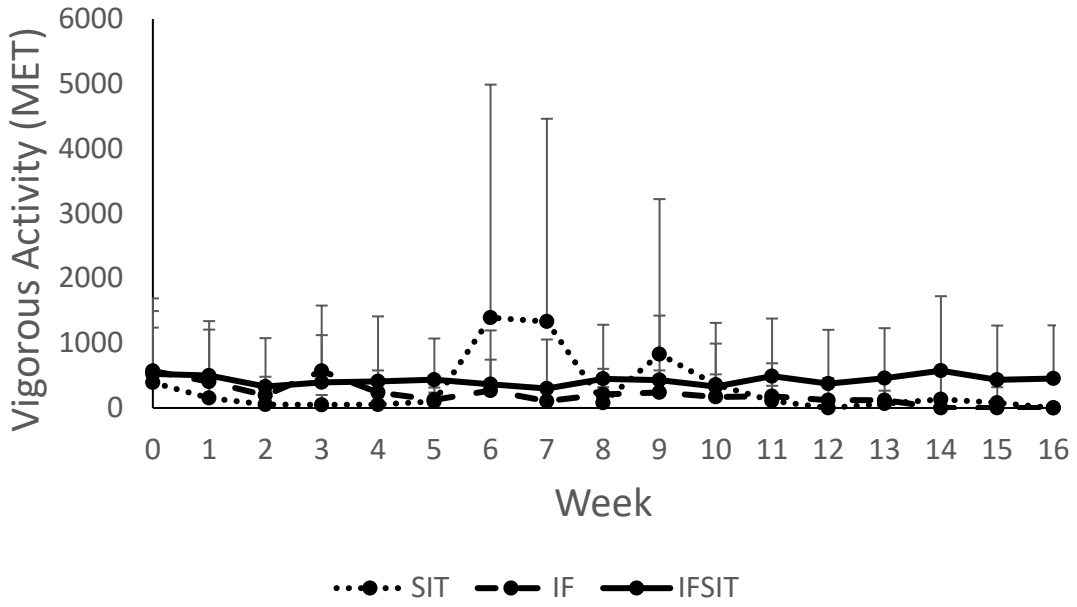


Figure 6.4 – *Mean changes to vigorous activity levels from baseline over a 16-week intervention period in participants undertaking 16 weeks of IF, IFSIT and SIT. Error bars represent ± S.D.*

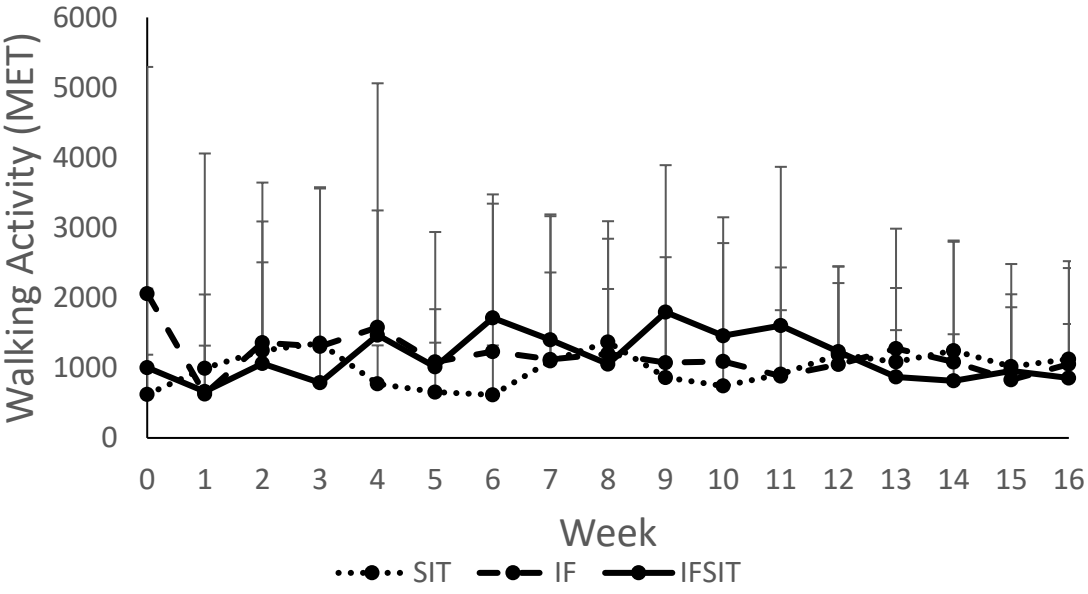


Figure 6.5 – *Mean changes to walking activity from baseline over a 16-week intervention period in participants undertaking 16 weeks of IF, IFSIT and SIT. Error bars represent ± S.D.*

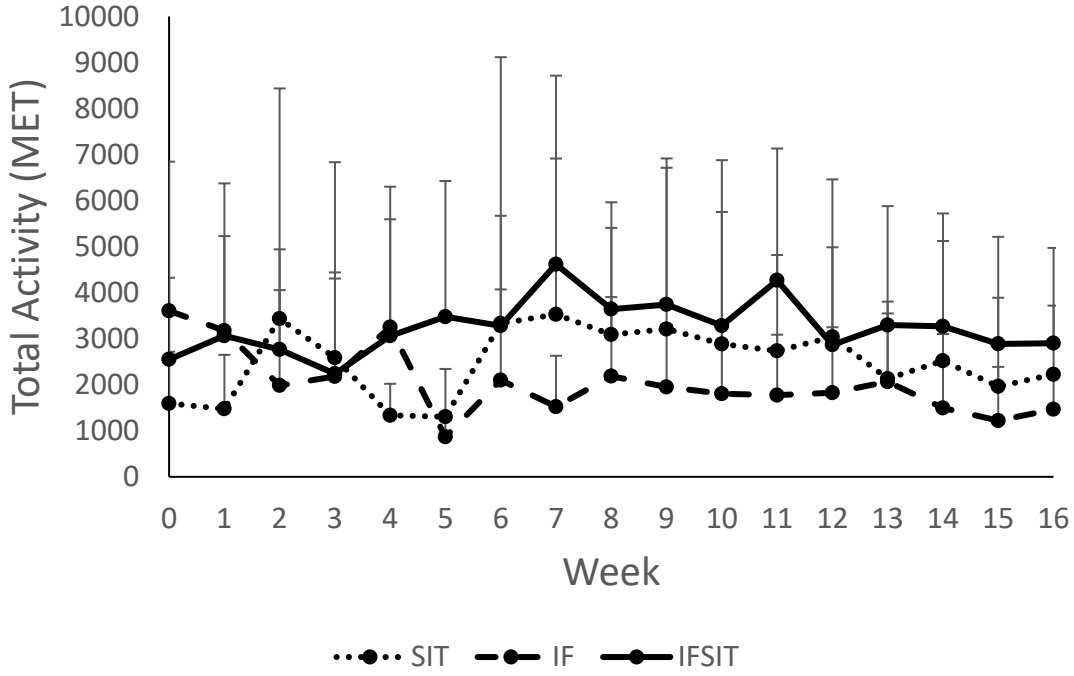


Figure 6.6 – *Mean changes to total activity levels from baseline over a 16-week intervention period in participants undertaking 16 weeks of IF, IFSIT and SIT. Error bars represent ± S.D.*

6.4 Discussion

In summary there was largely no change to the psychological and quality of life measures assessed during this study, seemingly refuting the hypothesis IF and SIT would provide improvements to mood state and quality of life measures and also refutes the hypothesis that there would be additive effect by combining the two protocols. Analysis of these data was confounded by relatively poor response rates to the questionnaires used. Profile of mood states data revealed a significant effect of time for confusion scores, with scores increasing over the course of the study. Additionally, there was a significant effect for time in total mental disturbance scores

with values decreasing in both fasting groups. Analysis of SF-36 data revealed that the IFSIT group reported significantly higher energy levels than the SIT group and a strong trend towards better general health than the SIT group, but with no other significant changes in the assessed parameters over the intervention period.

The activity data showed significant time and group*time effects in relation to sitting times, with sitting times increasing in both training groups but no other significant interactions

6.4.1 Psychological parameters & quality of life measures

In the current study there was little change in many of the parameters assessed using the profile of mood states questionnaire, with the exception of significant effects of time identified for both confusion and total mental disturbance (Fig. 6.1), with confusion scores increasing in all groups and total mental disturbance decreasing in both fasting groups. This contrasts with a similar study using a weekly 2-day fast over 4 months, which noted significant improvements in all parameters (Harvie *et al.*, 2013). Two other studies that also measured mood state and quality of life (albeit by alternate means) both reported improvements to mood state and energy levels over 8 weeks and 6 months respectively (Johnson *et al.*, 2007; Harvie *et al.*, 2011). It is possible that the more stringent interventions used in these studies was a factor, with Harvie *et al.* (2011 & 2013) using two consecutive days of fasting in their studies and Johnson *et al.* (2007) using *m*ADF. Additionally, as mentioned previously, a confounding factor in the current study was that there were several participants who experienced significant life events that negatively affected their mood state. Finally, the current study was carried out during the largest influenza outbreak in Australia since the 2009 pandemic (Australian Government, 2017). This led to multiple illnesses within the participant group over the study period and likely had a negative impact on the parameters assessed here.

Despite this the downward trend in TMD suggests that the fasting protocol used in this study was still able to produce a small positive impact on the participant's mood state despite the limitations experienced.

Quality of life was assessed by SF-36 questionnaire with no significant main effects or interactions identified, with only small changes over the 16-week intervention period. While few studies have investigated the effects of HIIT and SIT on quality of life, a study performed by Wisloff *et al.* (2007) reported improvements in quality of life measures after 12 weeks of high intensity interval training performed 3 times a week. Similarly, Johnson and colleagues (2007) were able to demonstrate improvements to quality of life, specifically in relation to emotional responses using IF, while Harvie *et al.* (2013) were able to demonstrate improvements in both the physical and emotional domains of the SF-36 questionnaire using IF. The reasons for this disparity between the literature and the lack of change reported here are likely similar to those relating to the POMS analysis.

6.4.2 Sitting time and activity levels

Sitting time increased from baseline in participants in the combined group and decreased in the SIT group with the difference between these groups approaching significance (p=0.059). Comparatively, sitting time was relatively unchanged in the IF only group (Fig. 6.3). Given the increasingly sedentary nature of the modern workplace with Australians spending 71-77% of the working day sitting or sedentary (De Cocker *et al.*, 2017) it is not surprising that sitting time didn't change markedly during this study.

Levels of moderate activity didn't change significantly during the study (Fig. 6.3) with no significant relationships detected despite relatively strong trends towards differences in group and group*time. Similarly, there was little change in participants levels of

vigorous activity over the study period (Fig. 6.4). Interestingly, however, within the SIT only group there were three weeks with greatly increased vigorous activity (weeks 6, 7 & 9), though the reasons for this are unclear. Levels of walking activity changed noticeably, albeit non-significantly, over the course of the study (Fig. 6.5) with the SIT and IFSIT groups generally increasing walking activity and the IF group generally decreasing their walking activity from baseline. Measures of total activity were relatively unchanged from baseline in the IFSIT and SIT groups (Fig. 6.6) with a trend towards decreased total activity in the IF only group.

Few studies have tracked physical activity during interventions pertaining to HIIT/SIT, with most using the IPAQ questionnaire as a screening tool for participant recruitment rather than as a tool for monitoring changes to physical activity, with accelerometers and other activity/heart rate trackers used for continuous monitoring outside of the prescribed exercise periods. A limitation of the current study was that funding was not available to enable the use of SenseWear™ or Fitbit™ devices to track activity.

In the studies that tracked participant activity over a number of weeks (8-12 weeks) there was no change in activity levels from pre- to post-intervention (Gillen *et al.*, 2014; Keating *et al.*, 2014). Similarly, the small number of IF studies that have tracked changes to activity levels have reported no significant changes using either SenseWear™ armband monitors or physical activity diaries (Harvie *et al.*, 2011; Kroeger *et al.*, 2012; Harvie *et al.*, 2013; Trepanowski *et al.*, 2017). From these studies it seems that the results obtained in the current study correlate well with what has been reported in the literature as the interventions did not appear to alter individual activity patterns over the 16-week intervention.

Chapter 7

General Discussion/Future directions

In general, the 5:2 diet was the more effective of the two protocols used with significant reductions in adiposity and metabolic parameters associated with obesity. Comparatively, SIT was effective for increasing exercise capacity and maintenance of lean muscle mass. There was no combined effect between the two protocols.

This study provides some evidentiary support the efficacy of the 5:2 intermittent fasting diet as marketed by Dr Michael Mosley and popularised in his television documentary entitled “Eat, Fast & Live Longer” and in his book “The Fast Diet” published in 2013 (BBC, 2012; Mosley & Spencer, 2013). This study is only the second to investigate the 5:2 diet in this form and the only study that has investigated its effects in a free-living population. For these reasons the current study contributes to the body of knowledge surrounding both sprint interval training and intermittent fasting in general, as well as providing information regarding the suitability of these protocols for use in an overweight or obese adult population both alone and in combination. There were 7 major findings relating to these factors:

1. **The 5:2 diet both alone and in combination with SIT has beneficial effects on body weight and composition in a free-living population, while SIT alone had little effect on these parameters. (Chapter 4)**

Following a 16-week intervention there were significant improvements in several parameters relating to body composition including significant decreases in body mass and BMI in both fasting groups when compared to SIT alone. There were also significant reductions in total fat mass in the IF only group and a comparable but non-

significant reduction in the IFSIT group in addition to non-significant reductions in several other parameters. These results taken together indicate that IF, both alone and in combination with SIT is an effective protocol for weight management and the reduction of excess fat mass in populations at risk of cardiovascular and metabolic disease with minimal dietary counselling and in the absence of pre-prepared meals.

SIT showed no benefit or effect on these parameters in either the SIT only group or when used concurrently with the 5:2 diet. It is possible that SIT training confounds these variables by altering muscle hypertrophy and other physiological effects that improve health. There is potential for future studies using HIIT training protocols (submaximal exercise) to investigate if these protocols are more suitable for use in obese and overweight populations due to the reduced intensity. Additionally, studies investigating meal timing and the prescription of macronutrient targets might also be warranted to elucidate whether these factors could increase the efficacy of the 5:2 diet on weight loss and in the preservation of lean body mass. Finally, future studies investigating the confounding factors that interfere with the combination of SIT with the 5:2 diet are also warranted.

2. **That the combination of the 5:2 diet and sprint interval training exacerbates the lean mass loss typically seen with continuous energy restriction and fasting strategies. (Chapter 4)**

This was the first study to report that the combination of SIT and IF leads to increased muscle catabolism. This is an interesting finding given that it might limit the suitability of this combination of interventions in some populations, particularly those in age groups suffering from sarcopaenia. While this study was not designed to determine the cause of this apparent synergy, it could be that limiting nutritional intake during periods of recovery following SIT could be counterproductive in terms of muscle remodelling.

This provides a potential route for future study when examining the use of these two protocols in combination to assess how combining SIT with IF negatively effects muscle remodelling and what the nature of these confounding factors are and how they can be overcome. Further, there is also potential for studies investigating the optimisation of lean mass retention when using intermittent energy restriction studies, as the LMM loss limits the widespread application of these protocols in older, at risk populations.

3. **The 5:2 diet, sprint interval training and the combined protocol did not significantly improve risk factors associated with cardiovascular disease despite a significant increase in aerobic capacity in both exercise groups. (Chapter 4)**

There were significant increases to absolute VO_{2peak} and VO_{2peak} when adjusted for both body mass and LMM between the IFSIT and IF groups and a strong trend towards increased absolute VO_2 between the SIT and IF groups reported in this study when compared to IF alone. However, these changes did not translate to significant improvements in other cardiovascular risk factors. There is a potential for studies focussed on optimising the cardiovascular benefit for participants by optimising dietary macronutrient content, fasting time and the activities that fasting strategies can be paired with. Additionally, studies examining non-fasting and post-prandial metabolic and cardiovascular changes would provide a more complete assessment of the acute effects of the 5:2 diet as these were not assessed in the current study.

4. **The 5:2 diet both alone and in combination with sprint interval training was more effective in decreasing serum leptin levels than sprint interval**

training despite little change in other metabolic health and inflammatory markers such as fasting glucose and HBA1c. (Chapter 5)

Hyperleptinaemia and loss of leptin sensitivity is a common symptom associated with obesity and the results obtained in the current study indicate that the 5:2 diet alone and in combination with SIT are effective at reversing hyperleptinaemia. This occurs as reductions in adiposity are linked to leptin levels, hence reduced fat mass leads to amelioration of hyperleptinaemia. These data also indicate a potential role in improving whole body sensitivity to leptin. Additionally, there were also non-significant decreases in other inflammatory, metabolic and oxidative stress markers. Further studies, with increased dietary counselling or with the provision of supplied/controlled meals are required to determine if the results obtained here were due to the free-living aspect of this study or if the 5:2 diet is ineffective in the improvement of glycaemic control and insulin resistance. Additionally, studies designed to optimise the application of the 5:2 diet in free-living populations are required as the current study shows that this is a limitation to the widespread application of fasting strategies in the general population.

5. **The 5:2 diet has little apparent effect on mood state, quality of life measures, satiety and attitudes to food. (Chapter 6)**

Another achievement of the current study is that we are the first study to report the effects of the 5:2 intermittent fasting diet on several psychological and lifestyle factors impacting participant's mental well-being and quality of life with few significant changes to these parameters observed during the intervention period. Further controlled studies with more stringent dietary intake reporting, dietary counselling and coaching are required to eliminate confounding variables and to provide a clearer determination on the effectiveness of these strategies on quality of life and mood state without the issues surrounding the investigation of these parameters in a free-living population.

Additionally, given that there was no evidence of negative effect on QoL and mood state, there is potential for further studies investigating the effects of the 5:2 diet on major depressive disorder and anxiety, particularly given the evidence supporting the benefits of weight loss on these conditions.

6. **The 5:2 diet and sprint interval training in isolation and in combination have no significant effect on caloric intake on non-fasting days, with the fasting groups exhibiting satisfactory levels of dietary compliance on fasting days, with no significant change to macronutrient intake over the intervention period. (Chapter 6)**

This finding supports the idea that weight loss with the 5:2 diet will occur in the absence of changes to non-fasting day caloric intake despite a noticeable but non-significant decline in energy intake in the IFSIT group. This point is often cited anecdotally as a reason that people choose the 5:2 diet over more stringent energy restriction and fasting protocols due to perceived ease of application. While this effect has also been noted in participants undertaking the alternate day fast, ours is the first study to report this in a free-living human cohort practising the 5:2 diet. Additionally, there were no significant changes to macronutrient intake noted in any of the intervention groups. As noted previously there exists an opportunity for future studies to be more prescriptive regarding macronutrient intake in an effort to increase weight loss, while optimising the retention of FFM.

7. **The 5:2 diet and sprint interval training alone and in combination have little impact on activity levels or time spent sitting outside of the exercise sessions prescribed by the study protocol. (Chapter 6)**

While not unexpected, it is still a major finding of this study that neither protocol, alone or in combination, was insufficient to significantly alter non-study related activity.

Limitations

The current study was subject to a number of limitations that influenced the data collection. The first of these was an inability to monitor activity levels in real-time. At the commencement of the study there was insufficient funding to acquire enough Fitbit™ or SenseWear™ activity trackers to record participant activity levels. In future studies we would ensure that these technologies were used as they have subsequently become much more affordable. The inclusion of activity monitors would remove the limitations of self-reported activity levels including overestimation and poor memory of past activity by monitoring changes in real-time. The second limitation was the sporadic nature of recruitment coupled with a higher than anticipated dropout rate, which led to difficulties with age-, BMI- and sex-matching of participants. This complicated data analysis as these issues led to a large discrepancy in the number of men vs. women in the study and led to significant differences in baseline characteristics. Due to sex-specific metabolic differences in the response to energy restriction this constitutes a serious limitation to the current study as it will limit the authors ability to disseminate the results of this study. Thirdly, the current study was carried out during the largest influenza outbreak in Australia since the 2009 pandemic. This complicated recruitment and inflated the dropout rate as several participants became ill during the study. Fourthly, allowing participants to answer the questionnaires in a self-guided fashion may have been a potential source of variation, as despite receiving instruction on how to answer the questions correctly, the participants may have applied their own interpretations to the questions rather than the intended interpretation. To correct this in future studies we would likely conduct these questionnaires over the telephone to

combat this, and to enhance the timely completion of the questionnaires as some participants would complete them up to half a week after they were due. Fifth, it was a limitation of the current study that the final meal before sampling wasn't controlled given the acute effects of macronutrient intake on metabolism. Additionally, it was a limitation of the current study that all metabolic measures were measured in a fasted state, rather than after food intake. IER has been demonstrated to effect post-prandial metabolism and studies examining all aspects of the 5:2 diet are needed to capture this information. Finally, another significant limitation to the current study is the absence of a control group. Due to the initial difficulties in recruitment, it was decided to use comparison groups rather than a dedicated control group particularly as it would be expected that there would be little to no change in the measured parameters in such within the control group. It should be noted, however, that many other studies in the field of intermittent fasting use similar arrangements, however a dedicated control group would have strengthened the study and the absence of one may negatively affect the dissemination of the results provided here.

Future Directions

Despite its limitations, the current study provides several avenues for future research. Firstly, given the absence of any negative effect on mental health during the study, coupled with the significant reductions in fat mass and body weight observed, there is scope for research investigating the effects of the 5:2 diet and SIT on major depressive disorder, anxiety disorders and other psychological conditions that may be improved through weight reduction and/or exercise. There is also scope for further studies that manipulate factors such as the timing and macronutrient content of the final meal before fasting and the use of different caloric intake restrictions on fasting days. Studies investigating these factors would provide much needed information relating to

the optimal range of calorie restriction for weight loss and the effect of macronutrient intake on fasting day satiety, weight loss and attitudes to fasting. Additionally, it would also allow optimisation of pre-fast meal timing. There also remains a need for further studies that investigate alternative exercise programs to cycling based SIT, particularly resistance-based exercise, to assess their effect on the lean mass retention, weight loss and their ability to improve participant attitudes to exercise. Additionally, studies focused on optimising the number of exercise bouts performed in each session and the duration of those bouts would be beneficial in selecting appropriate heart rate ranges to increase substrate utilisation. This could potentially be achieved by increasing the intensity and duration of bouts or by introducing more variety to the exercise program (e.g., providing a mix of resistance sessions and aerobic sessions). Finally, studies analysing other markers of stress and tissue function (adipose tissue, skeletal muscle etc.) would be beneficial and contribute greatly to our understanding of physiological adaptions to fasting, SIT and their associated effects on weight loss. While oxidative stress was measured in the current study using isoprostane levels this panel could be broadened to include other biomarkers of stress and health such as acute phase proteins, superoxide dismutase, ketone levels, liver enzymes, vascular cytokines, thermogenic factor uncoupling protein 1 (beige adipose tissue), myoglobin, troponin, malondialdehyde (fat peroxidation), heat shock proteins and glucocorticoid levels. Given the ease of obtaining suitable samples (e.g., blood and urine) many of these markers could add valuable information to the body of scientific knowledge.

Conclusions

In conclusion, this study is the first to investigate the possibility of synergism between the 5:2 diet and sprint interval training in humans and the first to investigate the efficacy and suitability for the application of the 5:2 diet in a free-living overweight and obese

population. Despite there being no evidence of synergism between the two protocols and only minor, non-significant improvements to several cardiovascular and metabolic risk factors there were significant improvements to total body mass, body composition and aerobic fitness. These effects indicate that diet is key for weight loss and that SIT is primarily involved in facilitating training adaptions such as increased aerobic capacity and increases in lean mass. Additionally, these effects indicate that SIT alone (without dietary changes) is insufficient to facilitate weight loss.

Chapter 8 - References

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Appendices

Appendix A – Food Diary

4 DAY FOOD DIARY

ID Number: _____

INSTRUCTIONS FOR FILLING IN FOOD DIARY

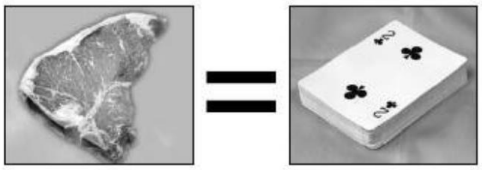
- **Record food and drink intake for 2 weekdays and the whole weekend.**

- DO NOT change your eating habits- we are interested in YOUR eating habits NOT the perfect diet.
- Please use a pen and write clearly.
- Record everything you eat and drink, right after it is eaten, including all snacks.
- Write each food or ingredient on a separate line.
- List all ingredients and their amounts for all food and drink.
- Under "Cooking method" briefly tell us how the recipe was prepared
- Use the measuring page 14 to measure some of your foods
- Fully describe foods and beverages, record brand names
- *Example:* Yoplait 97% fat free strawberry yoghurt
- **DO NOT** write 'steak" or 'bread'. Try to describe the food in detail:
- eg: T-bone steak without fat or bone
- Pre-sliced (Toast) wholemeal bread 'Country Split'
- **Explain how foods are prepared** - Example: Is meat fried or baked
- For foods prepared with fat, specify (butter, margarine, oil, etc.) and amount.
- Example: Fried in margarine (brand name), 2 TB
- For sandwiches, salads (mixed dishes), list each ingredient and amount.
  - Record exact amounts. Measure all foods in cups, teaspoons, table spoons, or size in cm. Use the ruler on back of this book, and the circles to measure circumference and thickness.
- When you are eating out, and are unable to use household measures use your body for comparison: REFER TO PAGE 2

Ways to Size Up Your Servings

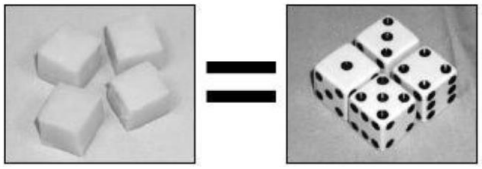
85 grams of meat is about the size and thickness of a deck of playing cards.

85g



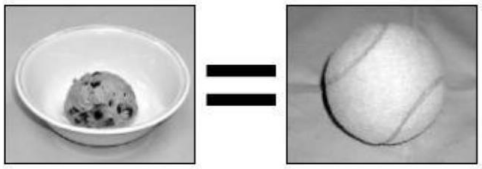
30 grams of cheese is about the size of 4 stacked dice.

30g



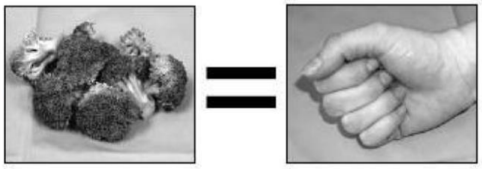
½ cup of ice cream is about the size of a tennis ball

½ Cup



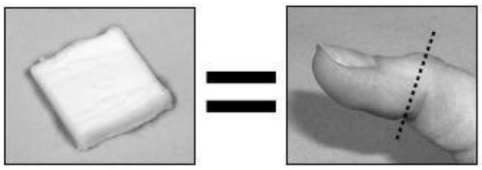
1 cup of mashed potatoes or broccoli is about the size of your fist.

1 Cup



1 teaspoon of butter or peanut butter is about the size of the tip of your thumb.

1 tsp.



Photos courtesy of: National Dairy Council, USA

Use comparisons- for describing portion sizes, it may be easier to describe portion sizes by comparing to objects eg. Potato the size of a hens egg, cheese- the size of a matchbox.

Weight on packaging- may also be useful in determining the amount eaten- eg. One buddy size coca cola= 600ml.

EXAMPLE

Please fill in as accurately as possible what you ate and the quantity for the past 24 hrs.

DAY OF THE WEEK: Tuesday **Date:** 03/5/2012

MEAL	FOOD/DRINK (With Brand)	QUANTITY (cups, spoons, number)	COOKING METHOD	FEELINGS/ REASON
EARLY MORNING	Coles low-fat Milk Cadburys drinking chocolate	1 glass 1 heaped tspn		Usual habit
BREAKFAST	Sultana Bran Coles low-fat Milk Tip Top White Bread (Toast sliced) Unsalted Butter Black earl grey tea	1 cup ½ cup 2 slices 1 heaped tbspn 1 cup	Toasted	hunger
MORNING TEA	Banana Nescafe instant coffee Coles Full Cream Milk Sugar- white	1 med- 15cm long 1 mug 2 tbspn 1 level tsp	Raw	Friend over
LUNCH	White bread roll Unsalted butter Virginian Ham Tomato Coca Cola	1 roll- matches circle 2 heaped tspn 3 large slices- 18 cm long, a thick 1 slice- Circle G, b thick 1 Can	Bakers Delight	hungry
AFTERNOON TEA	Didn't eat anything			
DINNER	Chicken stir fry see recipe 1. White Riesling wine	1 serve 2 wine glasses		It was dinner time
SUPPER	Chocolate self saucing pudding-white wings Peters vanilla ice cream	1 large serve- slice 10cm long 5 cm wide 6 cm thick ½ cup	Baked	Hungry while using computer
OTHER SNACKS	Tim-Tams Allens snake lollies	3 biscuits hand full		Boredom, watching tv

FOOD DIARY –Day 1

Please fill in as accurately as possible what you ate and the quantity for the past 24 hrs.

DAY OF THE WEEK:_____Date: _____/_____/_____

MEAL	FOOD/DRINK (With Brand)	QUANTITY (cups, spoons, no.)	COOKING METHOD	FEELINGS/ REASON
EARLY MORNING				
BREAKFAST				
MORNING TEA				
LUNCH				
AFTERNOON TEA				
DINNER				
SUPPER				
OTHER SNACKS				

FOOD DIARY –Day 2

Please fill in as accurately as possible what you ate and the quantity for the past 24 hrs.

DAY OF THE WEEK:_____Date: _____/_____/_____

MEAL	FOOD/DRINK (With Brand)	QUANTITY (cups, spoons, no.)	COOKING METHOD	FEELINGS/ REASON
EARLY MORNING				
BREAKFAST				
MORNING TEA				
LUNCH				
AFTERNOON TEA				
DINNER				
SUPPER				
OTHER SNACKS				

FOOD DIARY –Day 3

Please fill in as accurately as possible what you ate and the quantity for the past 24 hrs.

DAY OF THE WEEK: SATURDAY Date: _____/_____/_____

MEAL	FOOD/DRINK (With Brand)	QUANTITY (cups, spoons, no.)	COOKING METHOD	FEELINGS/ REASON
EARLY MORNING				
BREAKFAST				
MORNING TEA				
LUNCH				
AFTERNOON TEA				
DINNER				
SUPPER				
OTHER SNACKS				

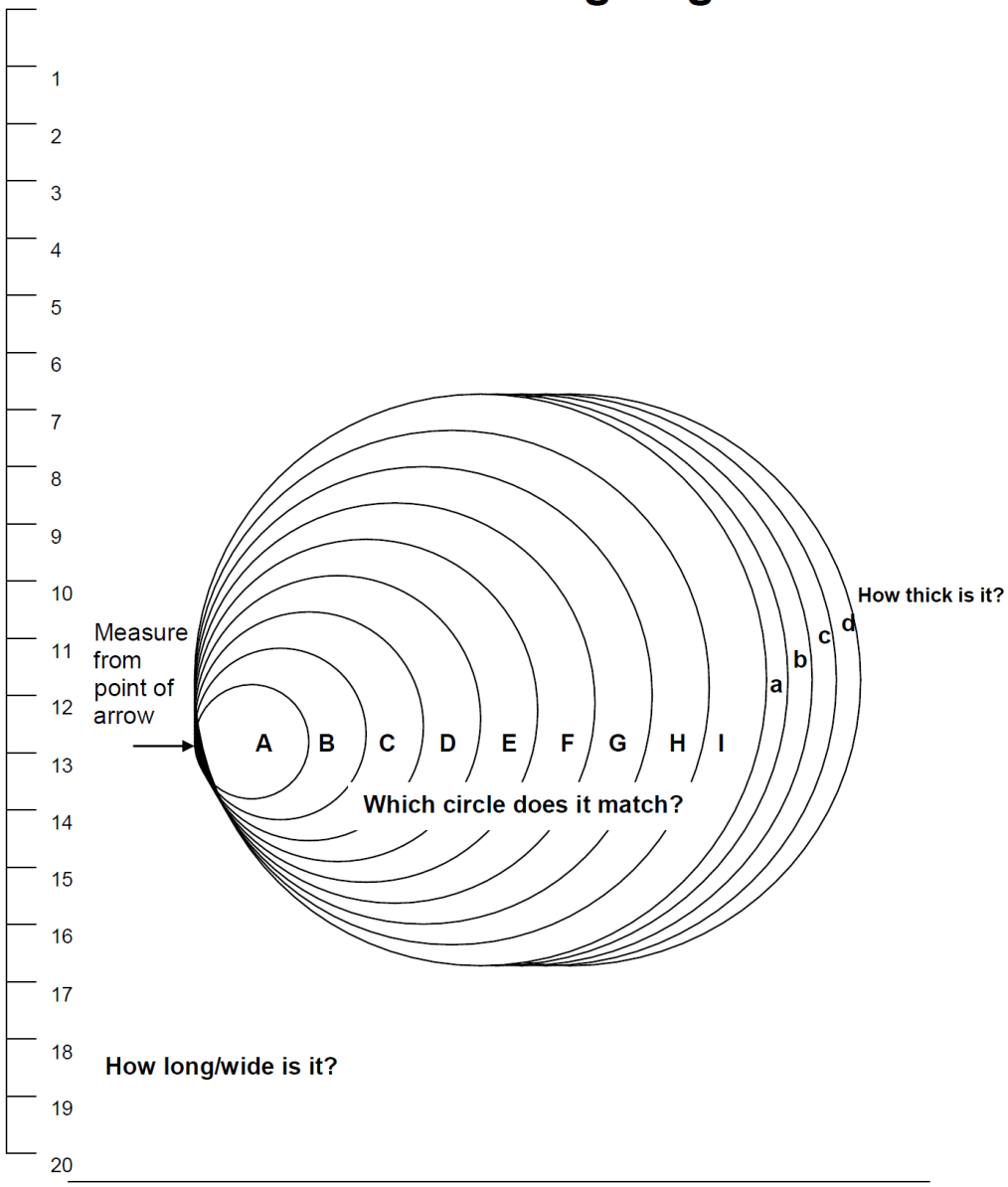
FOOD DIARY –Day 4

Please fill in as accurately as possible what you ate and the quantity for the past 24 hrs.

DAY OF THE WEEK: SUNDAY Date: _____/_____/_____

MEAL	FOOD/DRINK (With Brand)	QUANTITY (cups, spoons, no.)	COOKING METHOD	FEELINGS/ REASON
EARLY MORNING				
BREAKFAST				
MORNING TEA				
LUNCH				
AFTERNOON TEA				
DINNER				
SUPPER				
OTHER SNACKS				

The Measuring Page



8

Appendix B – Statistical Analyses

Table A4.1 – *Statistical analysis of body composition and anthropometric measurements performed by repeated measures ANOVA*

Time	*p* value	observed power	η^2	F
Body mass (kg)	0.000	0.995	0.348	16.558
BMI (kg/m^2)	0.000	0.997	0.369	18.093
Fat mass (kg)	0.000	0.999	0.352	16.810
Lean Mass (kg)	0.121	0.401	0.069	2.297
VAT (g)	0.001	0.949	0.207	8.068
IMAT (mm^2)	0.931	0.060	0.004	0.072
Hip:Waist ratio	0.022	0.681	0.126	4.469
Group	*p* value	observed power	η^2	F
Body mass (kg)	0.005	0.990	0.289	6.296
BMI (kg/m^2)	0.004	0.885	0.301	6.661
Fat mass (kg)	0.013	0.776	0.245	5.023
Lean Mass (kg)	0.057	0.561	0.169	3.146
VAT (g)	0.142	0.394	0.118	2.078
IMAT (mm^2)	0.774	0.085	0.027	0.260
Hip:Waist ratio	0.140	0.397	0.119	2.093
Group*time	*p* value	observed power	η^2	F
Body mass (kg)	0.004	0.901	0.260	5.446
BMI (kg/m^2)	0.002	0.922	0.273	5.813
Fat mass (kg)	0.003	0.911	0.237	4.826
Lean Mass (kg)	0.054	0.637	0.146	2.655
VAT (g)	0.148	0.508	0.102	1.765
IMAT (mm^2)	0.928	0.079	0.022	0.215
Hip:Waist ratio	0.168	0.445	0.101	1.734

Abbreviations: BMI: Body Mass Index, VAT: Visceral Adipose Tissue, IMAT: Intramuscular Adipose Tissue.

Table A4.2 – *Statistical analysis of cardiovascular measurements performed by repeated measures ANOVA.*

Time	*p* value	observed power	η^2	F
PBP Systolic (mmHg)	0.012	0.776	0.181	4.878
PBP Diastolic (mmHg)	0.136	0.406	0.091	2.095
CBP Systolic (mmHg)	0.016	0.702	0.196	4.642
CBP Diastolic (mmHg)	0.126	0.421	0.090	2.174
Heart Rate (bpm)	0.020	0.716	0.123	4.195
Pulse wave Velocity (m/s)	0.979	0.053	0.001	0.022
$VO_{2\ Peak}$ ($ml.kg^{-1}.min^{-1}$)	0.000	0.985	0.286	12.019
Absolute VO_2 ($ml.min^{-1}$)	0.204	0.320	0.052	1.646
$VO_{2\ Peak}$ ($ml.kg^{-1}LMM.min^{-1}$)	0.045	0.601	0.098	3.271
Total Cholesterol (mmol/L)	0.227	0.311	0.048	1.521
Triglycerides (mmol/L)	0.541	0.149	0.020	0.620
LDL Cholesterol (mmol/L)	0.175	0.351	0.057	1.815
HDL Cholesterol (mmol/L)	0.606	0.116	0.015	0.458
Group	*p* value	observed power	η^2	F
PBP Systolic (mmHg)	0.985	0.052	0.001	0.015
PBP Diastolic (mmHg)	0.717	0.097	0.031	0.338
CBP Systolic (mmHg)	0.632	0.116	0.047	0.470
CBP Diastolic (mmHg)	0.676	0.106	0.035	0.399
Heart Rate (bpm)	0.681	0.107	0.025	0.390
Pulse wave Velocity (m/s)	0.901	0.064	0.007	0.104
$VO_{2\ Peak}$ ($ml.kg^{-1}.min^{-1}$)	0.001	0.944	0.356	8.292
Absolute VO_2 ($ml.min^{-1}$)	0.012	0.788	0.257	5.185
$VO_{2\ Peak}$ ($ml.kg^{-1}LMM.min^{-1}$)	0.001	0.957	0.372	8.886
Total Cholesterol (mmol/L)	0.683	0.106	0.025	0.386
Triglycerides (mmol/L)	0.217	0.313	0.097	1.610
LDL Cholesterol (mmol/L)	0.989	0.052	0.001	0.011
HDL Cholesterol (mmol/L)	0.291	0.257	0.079	1.285
Group*time	*p* value	observed power	η^2	F
PBP Systolic (mmHg)	0.977	0.071	0.010	0.113
PBP Diastolic (mmHg)	0.838	0.123	0.033	0.356
CBP Systolic (mmHg)	0.906	0.099	0.026	0.253
CBP Diastolic (mmHg)	0.899	0.103	0.024	0.265
Heart Rate (bpm)	0.616	0.206	0.043	0.669
Pulse wave Velocity (m/s)	0.682	0.180	0.037	0.575
$VO_{2\ Peak}$ ($ml.kg^{-1}.min^{-1}$)	0.002	0.940	0.267	5.470
Absolute VO_2 ($ml.min^{-1}$)	0.009	0.856	0.207	3.912
$VO_{2\ Peak}$ ($ml.kg^{-1}LMM.min^{-1}$)	0.001	0.974	0.279	5.807
Total Cholesterol (mmol/L)	0.802	0.138	0.026	0.408
Triglycerides (mmol/L)	0.103	0.572	0.119	2.024
LDL Cholesterol (mmol/L)	0.993	0.057	0.003	0.050
HDL Cholesterol (mmol/L)	0.418	0.268	0.061	0.980

Abbreviations: PBP: Peripheral Blood Pressure, CBP: Central Blood Pressure: Low Density Lipoprotein HDL: High Density Lipoprotein

Table A5.1 – *Statistical analysis of cardiovascular measurements performed by repeated measures ANOVA.*

Time	*p* value	observed power	η^2	F
Fasting Glucose (mmol/L)	0.348	0.230	0.33	1.073
HBA1c (%)	0.407	0.201	0.030	0.914
Glucose Tolerance (AUC)	0.813	0.068	0.005	0.141
HOMA-IR	0.364	0.155	0.039	0.902
Insulin (µlU/ml)	0.456	0.177	0.039	0.802
IGF-1 (ng/ml)	0.423	0.142	0.035	0.751
Leptin (ng/ml)	0.014	0.764	0.185	4.767
Ghrelin (pg/ml)	0.359	0.221	0.048	1.051
TNF-α (pg/ml)	0.317	0.204	0.083	1.171
C-reactive protein (ng/ml)	0.094	0.475	0.107	2.504
Isoprostanes (ng/ml)	0.003	0.895	0.270	7.785
Group	*p* value	observed power	η^2	F
Fasting Glucose (mmol/L)	0.953	0.057	0.003	0.048
HBA1c (%)	0.676	0.108	0.026	0.397
Glucose Tolerance (AUC)	0.124	0.421	0.126	1.236
HOMA-IR	0.681	0.105	0.034	0.391
Insulin (µlU/ml)	0.367	0.208	0.095	1.053
IGF-1 (ng/ml)	0.380	0.203	0.088	1.014
Leptin (ng/ml)	0.065	0.538	0.229	3.126
Ghrelin (pg/ml)	0.294	0.250	0.110	1.297
TNF-α (pg/ml)	0.660	0.105	0.062	0.429
C-reactive protein (ng/ml)	0.944	0.058	0.005	0.058
Isoprostanes (ng/ml)	0.716	0.097	0.031	0.339
Group*time	*p* value	observed power	η^2	F
Fasting Glucose (mmol/L)	0.477	0.266	0.054	0.888
HBA1c (%)	0.314	0.358	0.075	1.214
Glucose Tolerance (AUC)	0.115	0.503	0.118	2.072
HOMA-IR	0.680	0.115	0.038	0.429
Insulin (µlU/ml)	0.578	0.214	0.068	0.728
IGF-1 (ng/ml)	0.367	0.239	0.093	1.078
Leptin (ng/ml)	0.043	0.700	0.205	2.710
Ghrelin (pg/ml)	0.046	0.691	0.202	2.659
TNF-α (pg/ml)	0.411	0.233	0.134	1.006
C-reactive protein (ng/ml)	0.848	0.120	0.032	0.342
Isoprostanes (ng/ml)	0.428	0.249	0.084	0.962

Table 6.1 – *Results of statistical analysis of profile of mood states data using repeated measures ANOVA over 16 weeks of intermittent fasting, sprint interval training or a combination of the two.*

Time	*p* value	observed power	η^2	F
Tension	0.221	0.438	0.055	1.456
Depression	0.977	0.067	0.004	0.094
Anger	0.526	0.232	0.030	0.786
Fatigue	0.139	0.493	0.069	1.839
Confusion	0.010	0.826	0.136	3.938
Vigour	0.260	0.362	0.052	1.358
Total mental disturbance	0.033	0.737	0.107	2.748
Group	*p* value	observed power	η^2	F
Tension	0.928	0.060	0.006	0.075
Depression	0.907	0.063	0.009	0.098
Anger	0.528	0.148	0.050	0.656
Fatigue	0.597	0.127	0.040	0.527
Confusion	0.306	0.245	0.090	1.243
Vigour	0.271	0.268	0.099	1.374
Total mental disturbance	0.438	0.179	0.069	0.855
Group*Time	*p* value	observed power	η^2	F
Tension	0.682	0.313	0.054	0.710
Depression	0.923	0.155	0.032	0.360
Anger	0.776	0.242	0.044	0.580
Fatigue	0.418	0.411	0.076	1.027
Confusion	0.361	0.426	0.082	1.116
Vigour	0.252	0.511	0.096	1.328
Total mental disturbance	0.894	0.195	0.037	0.441

Table A6.2 – *Results of statistical analysis of international physical activity questionnaire data using repeated measures ANOVA over 16 weeks of intermittent fasting, sprint interval training or a combination of the two.*

Time	*p* value	observed power	η^2	F
Sitting time	0.020	0.927	0.085	2.055
Moderate Activity	0.408	0.353	0.039	1.021
Vigorous Activity	0.436	0.226	0.035	0.902
Walking	0.688	0.215	0.024	0.611
Total Activity	0.504	0.273	0.033	0.850
Group	*p* value	observed power	η^2	F
Sitting time	0.073	0.517	0.212	2.958
Moderate Activity	0.099	0.462	0.169	2.546
Vigorous Activity	0.934	0.059	0.005	0.068
Walking	0.065	0.538	0.196	3.055
Total Activity	0.141	0.393	0.145	2.118
Group*time	*p* value	observed power	η^2	F
Sitting time	0.006	0.992	0.151	1.952
Moderate Activity	0.090	0.783	0.119	1.695
Vigorous Activity	0.312	0.420	0.088	1.214
Walking	0.182	0.684	0.102	1.416
Total Activity	0.288	0.563	0.089	1.226

Table A6.3 – *Results for the statistical analysis of weekly nutritional intake data using repeated measures ANOVA over 16 weeks of intermittent fasting, sprint interval training or a combination of the two.*

Time	*p* value	observed power	η^2	F
Average Calorie Intake	0.742	0.359	0.039	0.686
Avg. Fasting Day Calories	0.262	0.279	0.085	1.401
Avg. Carbohydrate Intake	0.281	0.595	0.067	1.228
Average Fat Intake	0.308	0.578	0.062	1.184
Average Protein Intake	0.710	0.119	0.021	0.409
Group	*p* value	observed power	η^2	F
Average Calorie Intake	0.206	0.291	0.158	1.598
Avg. Fasting Day Calories	0.118	0.343	0.155	2.757
Avg. Carbohydrate Intake	0.712	0.097	0.039	0.347
Average Fat Intake	0.336	0.222	0.114	1.159
Average Protein Intake	0.127	0.409	0.195	2.305
Group*time	*p* value	observed power	η^2	F
Average Calorie Intake	0.852	0.515	0.074	0.675
Avg. Fasting Day Calories	0.430	0.187	0.055	0.871
Avg. Carbohydrate Intake	0.494	0.680	0.103	0.973
Average Fat Intake	0.902	0.422	0.062	0.596
Average Protein Intake	0.145	0.546	0.154	1.735