Comparing the effects of 5:2 intermittent fasting and continuous energy restriction when combined with resistance training on body composition, muscular strength, cardio-metabolic health markers and dietary compliance.

Stephen Keenan

BFoodSc&Nutr, MDietetics

Faculty of Health, Arts & Design, School of Health Sciences,

Swinburne University of Technology,

Hawthorn, Victoria, 3122,

Australia

Presented for the degree of Doctor of Philosophy (Health Sciences)

November, 2020

Abstract

Background

Energy restricted diets are a common method for individuals to lose weight and body fat, in order to promote health and reduce risk of disease. While continuous energy restriction (CER) is commonly recommended as best-practice for inducing weight loss, these diets are difficult to comply with, and often have unwanted side-effects such as the loss of lean body mass (LBM). Intermittent fasting (IF) has been proposed as an alternative to CER that may promote better compliance, improve cardio-metabolic disease risk factors and be protective of LBM during weight loss. Nonetheless, energy restriction can still lead to LBM loss regardless of pattern of intake, and should therefore be combined with strategies known to promote LBM retention and growth (i.e. resistance training). Few studies have compared training-induced adaptations when resistance training is combined with either IF or CER, and none have utilised 5:2 IF, despite this mode being largely responsible for the recent surge in the popularity of IF.

Objectives

The objectives of this project were to investigate and compare the effects of resistance training when combined with either 5:2 IF, or CER with similar overall energy restriction and protein intake on body composition, muscle size, quality and strength, cardio-metabolic markers of health, dietary compliance and ratings of hunger, mood and energy levels.

Methods

A 12-week randomised dietary intervention was completed by 34 individuals with excess body fat. Participants were required to complete 3 resistance training sessions per week (2 supervised resistance training and 1 unsupervised aerobic/resistance training combination session), and were randomised to either a 5:2 IF [2 non-consecutive days consuming ~30% estimated energy requirements and 5 days consuming 100% of energy requirements (IFT)] or CER diet [daily consumption of ~80% estimated energy requirements (CERT)]. Both groups aimed for an intake of ≥1.4 grams of protein per kilogram of bodyweight. Outcome measures included assessment of body composition via dual x-ray absorptiometry (DXA), thigh muscle quality and size via ultrasonography and peripheral quantitative computed tomography (pQCT), strength via 3 repetition maximum and endurance testing on bench press and leg press, dietary intake via 3 day food records, cardio-metabolic markers via fasting serum samples and ratings of hunger, mood, energy levels and compliance via daily surveys.

Results

Both intervention groups lost weight and fat and gained LBM over the intervention period. There appeared to be a protective effect of fasting on LBM as magnitude of weight loss increased in the IFT group. However, there were discrepancies between measures of whole-body LBM and local muscle changes in the thigh, with the latter showing greater growth in the CERT group. Changes in cardio-metabolic markers were similar between groups, though the IFT group experienced greater reductions in total cholesterol and LDL-cholesterol than the CERT group, that may have been mediated by higher baseline values. Dietary compliance was high in both groups, while ratings of hunger and cravings were low at the beginning of the intervention, and further decreased over the 12 weeks. Participants indicated a willingness to continue with their diets after the intervention, albeit in modified form.

Conclusions

Both interventions led to beneficial changes in body composition. While there appeared to be a protective effect of 5:2 fasting on LBM as magnitude of weight loss increased, the

mechanisms underlying this are unknown, and this finding needs to be confirmed using larger sample sizes. Both diets led to generally favourable changes in serum markers of cardio-metabolic health, however may be more effective in individuals with poorer baseline values. Finally, both diets appear tolerable in the short-medium term, and in the longer-term if modified, and thus are likely to be practical options for individuals wishing to lose weight.

Acknowledgments

I'd like to thank my entire supervisory team. Associate Professor Regina Belski for helping me throughout what ended up being a long and windy journey from my beginnings at La Trobe University to my completion at Swinburne University. She has always been supportive and I have never had any doubt in my mind that she has always had my best interests at heart. Dr Matthew Cooke for the countless hours he spent reviewing my papers (even if I sometimes dreaded receiving the feedback) and saving me when I thought I would never complete a successful blood draw. Dr Sam Wu for his encouraging chats when I needed them.

Thanks to Associate Professor Doa El-Ansary for teaching me how to use the ultrasound and Dr Ebrahim Bani Hassan for helping me with the DXA scans, running the pQCT scans and the early mornings he put in to achieve this. Thank you to Formulite for providing all the required supplements for my study to go ahead.

Thanks to all the participants who took part in the study, I know you tried your best to comply with our intervention and I was glad to be a part of your lives for the months we spent together.

I'd like to thank Eddie for being a great mate throughout the journey. It was nice to have someone who had been through it all already to gauge how I was tracking, and to empathise with me when I complained. Thanks to dad for his support, both financial and emotional, and my brother for all his help. While I can't share this moment with mum, she will always be a big part of who I am, and anything I manage to achieve in life is a reflection on the influence she had on me. Finally, thanks to Jenny for always supporting me, encouraging me to undertake this journey in the first place, and for making sure I ate something real occasionally.

List of publications

Journal articles

Published

Keenan, S.; Cooke, M.B.; Belski, R. The Effects of Intermittent Fasting Combined with Resistance Training on Lean Body Mass: A Systematic Review of Human Studies. Nutrients 2020, 12, 2349.

Submitted for publication

Intermittent fasting and continuous energy restriction result in similar changes in body composition and muscle strength when combined with a 12 week resistance training program (submitted to Journal of Nutrition)

Planned for publication

The impact of 12 weeks of resistance training with 5:2 intermittent fasting or continuous energy restriction on cardio-metabolic health.

Self-reported dietary compliance is similar between individuals undertaking 5:2 intermittent fasting and continuous energy restriction over a 12 week period.

Conference Abstracts

Keenan, S, Cooke, M, Wu, S, Hassan, EB, Meyer, D, Chen, WS, Sullivan, J, Duque, G, Belski, R. The Effect Of Continuous Energy Restriction Vs Intermittent Fasting, With Resistance Training, On Lean Mass: 3082 Board #3 May 29 3:15 PM - 5:15 PM. Medicine & Science in Sports & Exercise. 2020;52(7S):846.

Presentations

The effect of 12 weeks of intermittent fasting and resistance exercise on lean body mass. Swinburne Building Bridges Conference. November 6th, 2019.

Declarations

I declare that this thesis:

1. Contains no material that has been accepted for the award to the candidate of any other degree or diploma, except where prior permission to do so has been received by the ADRD, and with due reference made about this in the text of the examinable outcome;
2. To the best of my knowledge contains no material previously published or written by another person except where due reference is made in the text, and with permission received to republish the work in the thesis; and,
3. Where the work is based on joint research or publications, I have disclosed the relative contributions of the respective creators or authors using the Authorship Declaration Form.

Stephen Keenan, 12th November, 2020

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List of abbreviations

ADF – alternate day fasting

BMR – basal metabolic rate

BMI – body mass index

CER – continuous energy restriction

CERT – continuous energy restriction plus resistance training group

CSA – cross-sectional area

DXA – dual x-ray absorptiometry

EI - echogenicity

HDL-C – high density lipoprotein-cholesterol

HOMA-IR – homeostatic model assessment of insulin resistance

hsCRP – high-sensitivity C-reactive protein

IF – intermittent fasting

IFT – intermittent fasting plus resistance training group

LBM – lean body mass

LDL-C – low density lipoprotein-cholesterol

MPS – muscle protein synthesis

pQCT – peripheral quantitative computed tomography

RF – rectus femoris

TC – total cholesterol

TRF – time-restricted feeding

VI – vastus intermedius

3RM – 3-repetition maximum

Chapter One

Chapter One: Introduction

This thesis investigates the effects of 2 different kinds of energy restricted diets combined with resistance training on a number of health-related variables, while also exploring the ability of individuals to comply with these diets. The 2 diets explored are continuous energy restriction (CER), and a version of intermittent fasting (IF) known as 5:2 IF. This chapter provides a brief introduction to these two dietary methods, while also discussing the importance of pairing energy restriction with resistance training and an adequate protein intake. It also gives a rationale for exploring these diets and provides an overview of the subsequent chapters in this thesis.

1.1 The problem with dieting

The rise in prevalence in overweight and obesity is a reflection of changing dietary and behavioural patterns, with eating habits shifting towards greater consumption of energy dense foods, and decreasing levels of physical activity. Two thirds of Australian adults are considered overweight or obese, and more than half are considered insufficiently active (1). While the health risks of having a body mass index (BMI) over the healthy range of 18.5-24.9kg/m^2 are well known, these risks are likely to be partially related to excess adiposity rather than simply being overweight (2). As BMI is a blunt tool, designed to be used for population level comparisons, it is unable to identify individuals who may be overweight due to large amounts of lean body mass (LBM) despite low fat mass, and conversely, it is unable to identify individuals within the healthy BMI range who possess excess levels of body fat. These latter individuals are considered to have 'normal weight obesity', and this poses similar cardio-metabolic risks to being overweight or obese as measured by BMI (3).

Nonetheless, the loss of weight and body fat in individuals who are overweight, obese and those with normal weight obesity may lead to improvements in disease risk markers, and the most popular effective way to achieve this is through weight loss via an energy restricted diet and increases in physical activity (4). However, there are a number of pit-falls associated with energy restriction. First, while the main purpose of energy restriction is to reduce body fat, a common side-effect is the loss of LBM, which has ramifications for appearance, physical function (5) and leads to decreases basal metabolic rate (BMR) (6) that may increase the risk of future weight re-gain. Second, it isn't easy. Indeed, compliance with energy restricted diets is notoriously poor (7), which may be caused in part by increases in appetite and hunger, and the mental burden associated with dieting (8). Third, even if individuals succeed at losing weight, the challenges associated with maintaining weight loss are significant, and rates of weight recidivism are less than ideal. Unfortunately, this recidivism has implications for health, as weight regain may be comprised of proportionately more fat mass than LBM compared to the weight that what was initially lost (9,10), an effect that may be even more pronounced in normal weight individuals (11). This would lead to a situation where individuals may end up back at, or close to their baseline weight, but with even greater amounts of adipose tissue. This weight regain is made more likely by the fact that during weight loss, BMR decreases disproportionately more than would be expected based on losses of LBM, an effect that remains even through extended periods of weight maintenance (12,13). Additionally, when weight is regained, BMR does not increase in proportion to the mass regained (14), which is likely to inhibit future weight loss attempts.

It is obvious that there are significant challenges associated with losing weight that extend beyond the weight loss phase. Unfortunately, despite decades of research, a lifestyle focused weight loss intervention that is effective at producing and maintaining high quality weight loss has remained elusive. Nonetheless, with more than 2.5 billion people either overweight or obese around the world (a number that does not include those with normal weight obesity) (15), researchers continue to search for a sustainable method of weight and fat loss.

Although the above discussion could lead one to think that attempts at weight and fat loss are futile, there are ways to minimise at least some of the adaptive responses seen during weight loss. These strategies largely focus on methods of preserving LBM during weight loss, thus reducing the effect on energy expenditure related to loss of this tissue. One way of doing this is adjusting how quickly weight is lost. The amount and speed of weight loss is dependent on the magnitude of the energy deficit. While greater energy deficits (for example through very-low-calorie diets) lead to more rapid weight loss, they are also associated with greater LBM loss (16,17), and therefore greater reductions in BMR. They may also lead to more pronounced adaptive responses (reductions in energy expenditure beyond that accounted for by LBM) that favour weight re-gain, at least during the weight loss phase (18). Conversely, while less severe energy deficits and slower rates of weight loss do tend to improve outcomes with regards to LBM (16,17,19), they also require compliance over longer-time periods, which may theoretically make them more difficult. Nonetheless, more moderate energy restrictions are commonly recommended for weight loss, in hope that they might promote long-term maintenance. Consuming protein over and above recommended daily intakes has also been shown to help promote LBM maintenance during weight loss (20) and is commonly prescribed alongside energy restriction. Finally, the addition of exercise to energy restriction has been shown to not only reduce the amount of weight lost as LBM, but also in some instances attenuate the additional reductions in energy expenditure seen with weight loss (18). In particular, resistance training promotes greater LBM preservation due to its impacts on muscle protein synthesis (MPS).

While these strategies do not guarantee that weight loss will be maintained, it is important that interventions that induce weight loss focus on optimising the quality of that weight loss (that is, greater proportions of weight lost as fat while LBM is preserved or accrued) by implementing these strategies, while also paying close attention to the tolerability of these interventions, as this may assist with long-term maintenance.

1.2 Enter intermittent fasting

Energy restricted diets come in many forms (e.g. low-fat, low-carbohydrate, high-protein etc.), however most commonly the underlying principle is a daily energy deficit; that is, reducing energy intake to below energy requirements every day. This form of energy restriction (CER), is the method generally recommended by governing health bodies for managing overweight and obesity (21). The majority of past weight-loss research has focused on CER, and thus, the limitations of energy restriction discussed above are largely based on this type of diet. However, interest in alternative methods of restriction has increased recently.

Fasting for religious reasons has been practiced for centuries, while the use of fasting for medical purposes also has a rich history, with a number of studies in the 20th century utilising extended fasts for the purposes of improving health. In 1965, Angus Barbieri set the Guinness World Record for the longest complete fast, when he underwent a medically supervised complete fast for a total of 382 days, reducing his body weight by a staggering 125 kg (22)! Amazingly, this weight loss was almost completely maintained 5 years later. While the authors of this study thanked Mr Barbieri for his 'cheery co-operation', they also recommended that extended starvation therapy be used cautiously, given that studies utilising this method had also led to 5 fatalities in the lead up to Mr Barbieri's attempt.

While such studies are unlikely to pass ethics in the modern era, a much less severe version of fasting for weight loss, IF, has recently gained popularity amongst both the general population and the scientific community, especially over the past 10 or so years. This type of energy restricted diet differs from CER in terms of timing of energy consumption. Whereas CER is characterised by a daily energy deficit, IF intersperses severe or complete energy restriction with periods of relatively greater intake. The most common forms of IF are: complete alternate day fasting (ADF); modified ADF, time-restricted feeding (TRF) and; 5:2 fasting. The differences in these fasting methods are described in Table 1.1.

Table 1.1. Variations of Intermittent Fasting

Type of fasting	Description of method
Alternate day fasting (ADF)	Alternates days of complete restriction (no energy intake) with days of either ad-libitum, euenergetic or less severe energy restriction.
Modified ADF	Alternates days of severe, but not complete, restriction (generally involving consumption of ~25% of energy requirements) with days of either ad-libitum, euenergetic or less severe energy restriction.
Time-restricted feeding (TRF)	Eating is restricted to a certain time period each day, generally between 4-8 hours, creating an extended fast each day.
5:2 fasting	Generally consists of 2 fasting days (consecutive or non-consecutive) each week that are restricted either completely (no energy intake) or severely (intake of ~25% of energy requirements). The remaining 5 days involve either ad-libitum, euenergetic or less severe energy restriction.

IF has been proffered as an alternative to CER that may promote greater compliance. The reasoning behind this, is that individuals do not need to focus on restricting their energy intake every day, but only for select time periods each day or a few days each week. While the overall energy restriction prescribed is similar to that of CER, this may help reduce some of the pre-occupation with food that occurs during restrictive dieting, and could theoretically reduce the mental burden on individuals trying to lose weight. Furthermore, some have proposed that IF may promote the maintenance of LBM during periods of weight loss, given the frequent reprieve from energy restriction. While some forms of IF have been compared to CER in terms of compliance, LBM and other markers of health, the theoretically infinite variability of IF patterns means that there may be a certain number of fasting days or severity of restriction that could lead to better outcomes than others. Furthermore, while research is growing, there are few studies that have combined IF with resistance training, despite its known capacity to protect or grow LBM during energy restriction. Thus, there remains a number of unanswered questions with regards to IF in relation to weight loss.

1.3 Overview of thesis

The remaining chapters of this thesis will: i) explore the current state of the literature on IF with regards to body composition, compliance and cardio-metabolic health, identifying gaps in the literature that still need to be addressed, and ii) detail the results of a randomised intervention study comparing the effects of resistance training combined with either 5:2 IF or CER. An overview of the remaining chapters is provided below.

Chapter 2 – Chapter 2 comprises 4 parts. The first is a systematic literature review reporting on the effects on LBM and overall body composition when resistance training is combined with IF. This has been published in the Journal *Nutrients*. The second part provides a brief discussion on theoretical mechanisms of IF that may be conducive to maintaining LBM and promoting fat loss. Part 3 is a discussion on the current state of the literature on the effects of IF on cardio-metabolic markers, while Part 4 details studies that have reported on how IF affects dietary compliance and participant ratings of mood and hunger.

Chapter 3 – Chapter 3 builds on chapter 2 by detailing the research questions arising from the identified gaps in the literature.

Chapter 4 – Chapter 4 provides a detailed methodology utilised in the randomised intervention study than was run as part of this PhD, that aimed to address the research questions listed in chapter 3. This includes details on dietary methodology, and all assessment outcomes. To reduce duplication, details of the methods used have been removed from subsequent chapters that have been written in the form of publishable manuscripts.

Chapter 5 – Chapter 5 reports on the changes in whole-body body composition, local thigh musculature and strength found in response to 12 weeks of resistance training combined with 5:2 IF or CER.

Chapter 6 – Chapter 6 describes changes seen in cardio-metabolic markers as a result of the intervention.

Chapter 7 – Chapter 7 reports levels of compliance to each diet, and the impacts of each diet on measures of hunger, cravings and mood.

Chapter 8 – Chapter 8 is a discussion of, and conclusion to the overall findings of this thesis. This chapter also includes a short section on potential future research directions to help further elucidate any differential effects of IF and CER.

Chapter Two

Chapter Two: Literature review

2.1 Preface

This chapter includes a systematic literature review of the impacts of IF combined resistance training on body composition. This literature review reveals a paucity of data with regards to this combination outside of TRF. This first section has been published in the journal *Nutrients*, and while it has been re-formatted for consistency with the rest of the thesis, is worded exactly as published (aside from supplementary material being re-worded to refer instead to appendices). Referencing is also in the style required by the journal. An authorship declaration form signed by all authors can be found in Appendix 1. I warrant that I have obtained, where necessary, permission from the copyright owners to use any third party copyright material reproduced in the thesis (such as artwork, images, unpublished documents), or to use any of my own published work (such as journal articles) in which the copyright is held by another party (such as publisher, co-author). A copyright statement from the relevant journal can be found in Appendix 2.

In the subsequent sections, a brief discussion is provided on some possible mechanisms of IF that may promote fat loss and protect LBM, and into the effects of IF on cardio-metabolic health, rates of compliance to IF compared to CER, and measures of mood and hunger.

2.2 Systematic literature review: the effects of intermittent fasting combined with resistance training on lean body mass (published in *Nutrients*)

Publication Details: **Keenan, S**., Cooke, M. B., & Belski, R. (2020). The Effects of Intermittent Fasting Combined with Resistance Training on Lean Body Mass: A Systematic Review of Human Studies. *Nutrients*, 12(8), 2349.

The Effects of Intermittent Fasting Combined with Resistance Training on Lean Body Mass: A Systematic Review of Human Studies

Stephen Keenan *, Matthew B. Cooke and Regina Belski

School of Health Sciences, Swinburne University of Technology, Hawthorn, VIC 3122, Australia; mbcooke@swin.edu.au (M.B.C.); rbelski@swin.edu.au (R.B.)

* Correspondence: skeenan@swin.edu.au

Received: 10 July 2020; Accepted: 03 August 2020; Published: 06 August 2020

Abstract: Diets utilising intermittent fasting (IF) as a strategic method to manipulate body composition have recently grown in popularity, however, dietary practices involving fasting have also been followed for centuries for religious reasons (i.e., Ramadan). Regardless of the reasons for engaging in IF, the impacts on lean body mass (LBM) may be detrimental. Previous research has demonstrated that resistance training promotes LBM accrual, however, whether this still occurs during IF is unclear. Therefore, the objective of this review is to systematically analyse human studies investigating the effects of variations of IF combined with resistance training on changes in LBM in previously sedentary or trained (non-elite) individuals. Changes in body weight and fat mass, and protocol adherence were assessed as a secondary objective. This review followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines. MEDLINE, CINAHL, PubMed and SportDiscus databases were searched for articles investigating IF, combined with resistance training that reported measures of body composition. Eight studies met the eligibility criteria. LBM was generally maintained, while one study reported a significant increase in LBM. Body fat mass or percentage was significantly reduced in five of eight studies. Results suggest that IF paired with resistance training generally maintains LBM, and can also promote fat loss. Future research should examine longer-term effects of various forms of IF combined with resistance training compared to traditional forms of energy restriction. Prospero registration CRD42018103867.

Keywords: intermittent fasting; exercise; resistance training; body composition; lean body mass

1. Introduction

Non-linear dieting approaches, such as intermittent fasting (IF), have recently gained popularity as a method of manipulating body composition. Variations of IF include total alternate day fasting (ADF), modified ADF, time-restricted feeding (TRF) and diet breaks, amongst others [1]. Whilst research into how they affect body composition is relatively recent, fasting for religious reasons has been practiced for centuries. Each year, millions of Muslims around the world participate in the holy month of Ramadan, abstaining from food and fluid from sunrise to sunset [2]. Regardless of the reason or method of fasting, all involve extended periods of time with little or no nutritional intake. While there are many purported health benefits with fasting, including those linked to elevated BMI and/or chronic inflammation [3–6], a potentially unfavourable consequence of energy restriction is loss of lean body mass (LBM). The maintenance of skeletal muscle mass (which is a large component of LBM), is determined by changes in rates of skeletal muscle protein synthesis (MPS) and skeletal muscle protein breakdown (MPB) [7]. Both MPS and MPB rates are influenced by energy balance. During energy balance, acute periods of positive and negative protein balance as a result of a fed and fasted state, respectively, have little impact on muscle mass as the net protein balance is neutral [8]. However, during energy restriction or fasting, rates of MPB might be accelerated and rates of MPS reduced [9,10]. Subsequently, this can result in a decline in net protein balance and may contribute, in part, to reductions in LBM.

Preservation or growth of LBM is important for a number of reasons. Firstly, given that LBM is a major determinant of basal metabolic rate (BMR), losses in LBM could have an important impact on total energy expenditure. For each kilogram of LBM lost, an estimated reduction in BMR by about 13 kcal per day has been reported [11], though estimates vary from 3–4 kcal, up to 33 kcal per kilogram [12,13]. While the amount of reduction in BMR seems trivial, especially at the lower ends of these estimates, the continued loss of LBM and reductions in BMR could subsequently compromise the efforts of individuals seeking to lose weight, and conversely, may increase the risk of weight gain. This may be further compounded by the possibility that losses of LBM may lead to increased hunger, thus also affecting the energy intake side of the energy balance equation (11,23)[a]. In addition, given LBM is a highly metabolically active tissue, reduced LBM may compromise aspects of metabolic health and increase the risk of disease development (i.e., type 2 diabetes) in overweight and obese individuals and/or may contribute to the development of sarcopenia and bone loss, especially in older adults [14,15]. Lastly, while not uniformly correlated [16], losses of LBM may compromise strength and physical function, with some studies reporting associated decreases in handgrip and knee-extensor strength [17]. For these reasons, strategies that prevent the loss of LBM as a result of diets incorporating fasting should be considered.

[a]Additional information for thesis, not in Keenan et al. (2020). References can be found in the final thesis reference list.

One such strategy that can attenuate LBM losses and could even result in gains in LBM during energy restriction is performing physical activity, specifically resistance training [18,19]. While the majority of research has focused on continuous energy restriction (CER), a number of emerging studies have investigated the impact of other forms of energy restriction, such as IF, combined with resistance training on body composition, including changes in LBM. However, to date, these studies have not been systematically reviewed. Therefore, the purpose of this systematic review is to assess the combined effects of IF (including Ramadan fasting) with resistance training on changes in LBM in sedentary and active (non-elite), overweight and normal weight individuals. The secondary objective is to assess the effects of the same diet intervention protocols on body fat, weight and adherence rates. This review was conducted and reported according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [20].

2. Materials and Methods

This study was conducted and reported according to the PRISMA guidelines [20]. The PRISMA checklist can be found in Appendix 3. A protocol was designed a priori and registered with PROSPERO, registration number = PROSPERO 2018 CRD42018103867 [21].

2.1. Data Sources

A systematic search was conducted using the databases MEDLINE, CINAHL, PubMed and SportDiscus for original research articles published in peer reviewed journals that had investigated the impact of IF combined with resistance training on body composition. Key search terms were: alternate day fast* OR alternat* calori* diet* OR alternate day diet* OR alternate day modified fast* OR intermittent fast* OR intermittent energy fast* OR intermittent energy restrict* OR intermittent calori* restrict* OR ADF OR time restricted feed* OR TRF OR Ramadan OR Ramadan fast* AND exercis* OR resistance exercis* OR training AND body composition. An example of a full electronic search strategy in MEDLINE can be found in Appendix 4.

2.2. Study Selection

No restrictions were placed on publication date, as research in this area is relatively recent and limited in amount. To be included in this review, studies were required to meet the following criteria: (i) original article; (ii) interventions in humans; (iii) include a description of the fasting protocol; (iv) include a description of the exercise undertaken by participants during the trial period, and include a resistance training component; (v) include a measure of body composition and report on either body fat (as a percentage or as total fat mass) or lean body mass. Exclusion criteria included: (i) non-human studies; (ii) reviews, case studies, letters, surveys, abstracts, conference papers or duplicate reports; (iii) non-English publication; (iv) grey literature; (v) studies in elite or professional athletes. Papers were not excluded for lack of control group or comparison group, as long as they met the above inclusion criteria. Final searches were performed on 16 July 2018 and updated on 17 April 2020.

2.3. Data Extraction

The initial database search resulted in 243 studies being returned. Of these, 36 met the inclusion criteria and underwent full-text review. The reference lists of these studies were searched for further appropriate studies, with 3 more being identified for full review. Out of these 39 studies, 8 met the inclusion criteria. From these studies, publication data, participant details, duration of study, description of fasting method and exercise protocol, mean weight and anthropometric changes, methods of anthropometric assessment and adherence rates were extracted into a custom template. The PRISMA flow diagram [20] for this search process, including reasons for study exclusion, is presented in Figure 2.1.

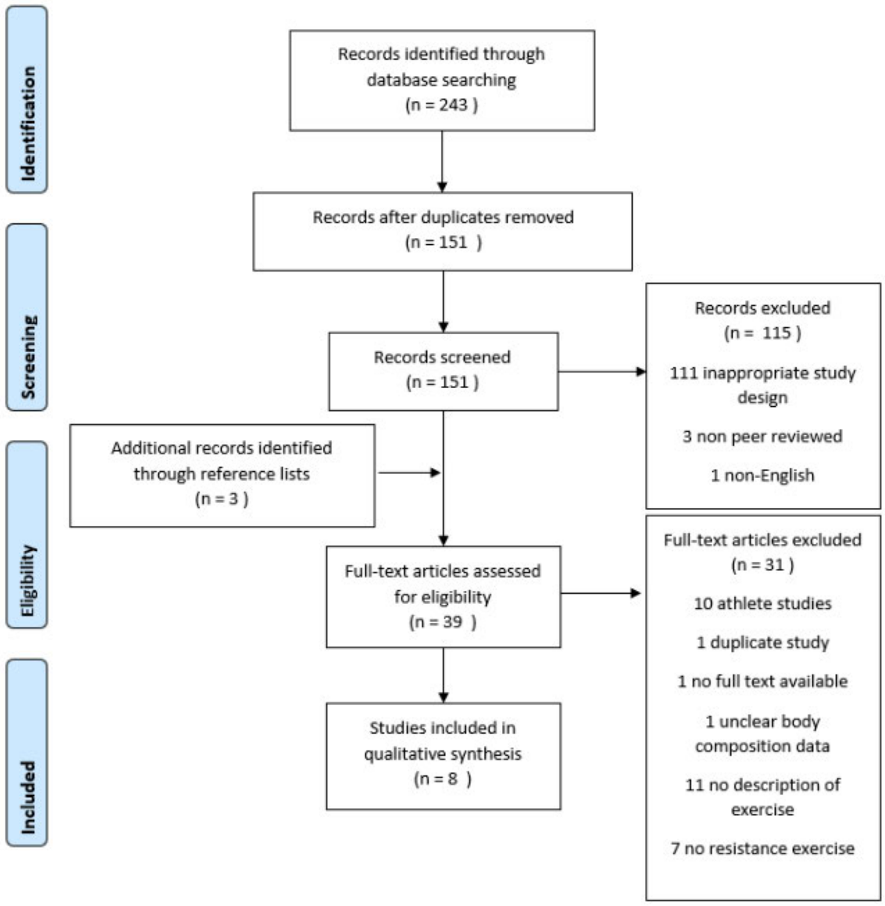

Figure 2.1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of study selection.

2.4. Quality Appraisal

The Downs and Black quality check list [22] was used to assess the risk of bias in each study consistent with previously published reviews in this field [23]. This checklist comprises 27 questions addressing reporting quality and external and internal validity. Question 14, regarding blinding of subjects, was removed as this is not seen as possible in these dietary interventions. Each question is rated as either a yes (1 point), no (0 points) or unable to determine (0 points), with 1 question rated on a 3 point scale (yes = 2 points, partial = 1 points, no = 0 points). Given varying primary outcomes of the studies included, and the difficulty of assigning a clinically meaningful difference for these outcomes, the final question relating to power analyses has been altered. Studies were awarded 1 point if they included sample size calculations, and no points if this was omitted. Maximum scores of 27 and 26 were available for randomised and non-randomised studies, respectively.

3. Results

Participant and study characteristics are presented in Table 2.1 [24–31].These include: body mass index (BMI), age, sex, number of drop outs, length of intervention period, description of fasting and exercise protocol, weight and body composition changes (effects of the intervention), method of anthropometric assessment and study quality score. Additionally, information on adherence to dietary protocols was also included in the body of this review from studies that reported it.

3.1. Intervention Period

Intervention periods ranged from 4 to 8 weeks. Three studies that investigated Ramadan fasting were approximately 4 weeks in duration [29–31]. Three studies that investigated TRF were 8 weeks in duration [24–26], while a fourth was 4 weeks in duration [27]. The one study that investigated modified ADF was 8 weeks in duration [28].

3.2. Participant Characteristics

Of the eight studies included, there were a combined total of 219 participants across all groups, including 153 males and 66 females. Twenty-seven individuals withdrew from their respective studies [25–28], leaving 192 individuals completing the interventions. A total of

120 individuals completed the IF intervention arm and 72 completed the comparison arms. The sex distribution of final numbers of completed participants could not be accurately determined, given that one study did not report the sex of withdrawn participants [28]. Eight participants from two studies were excluded from analysis due to non-compliance [25,27]. Previous exercise/training history was varied between studies, with some participants classified as recreationally active [25,31] or resistance trained [26,27,29,30]. One study provided insufficient details regarding previous exercise/training history, though it was noted participants were unfamiliar with resistance exercise [25]. The age of the participants ranged from young adults (22.0 ± 2.4 year) [25] to middle-aged adults (40.6 ± 10.0 year) [28]. BMI ranged from normal weight (22.5 ± no SD) [26] to moderately overweight (28.3 ± 4.1) [28]. Only three studies reported the BMI of participants [28–30]; the BMI of the remaining studies was calculated using mean baseline height and weight values. Baseline body fat percentage ranged from 12.9 ± 3.5% [31] to 34.9 ± 4.6% [28], though it should be noted that various assessment techniques were used.

3.3. Intervention—Fasting Protocol

Of the eight studies reviewed, three studies investigated Ramadan fasting [29–31], with fasting durations approximately 14–15 h per day. Only one of these studies included a control diet group [30], while the remaining Ramadan studies reported no control diet group. Four studies utilised TRF [24–27]. Of those, one study instructed participants to consume their daily intake within a 4 h window between 4 p.m. and midnight, 4 days per week, with no restrictions on food choices or overall intake [25]. On the other 3 days per week, participants were allowed ad libitum intake. Three studies instructed participants to consume all of their daily intake within an 8 h window [24,26,27]. The first of these involved participants consuming 100% of their estimated energy requirements over three regular intervals at 1 p.m., 4 p.m. and 8 p.m. every day [24]. The second study allowed intake between 12 p.m. and 8 p.m. without set intervals, aimed for participants to be in a small energy deficit (~250 kcal), and to consume a high protein diet (≥1.4 g of protein per kilogram of bodyweight per day) [26]. This study also included another TRF group with the same guidelines, but which consumed an additional 3 g of β-hydroxy β-methylbutyrate (HMB) per day. The third study allowed participants the choice of consuming their energy intake between 12 p.m. and 8 p.m., or 1 p.m. and 9 p.m. [27]. Participants aimed to restrict energy by approximately 25% of estimated requirements, and also consume 1.8 g of protein per kilogram of bodyweight per day. Comparison diet groups included: usual dietary intake with no time restrictions or scheduled times of consumption [25]; usual dietary intake, but consumed within a 13 h period and scheduled times of consumption (8 a.m., 1 p.m. and 8 p.m.) [24]; eating at self-selected intervals but with the same 250 kcal energy deficit and a protein intake of ≥1.4 g of protein per kilogram of bodyweight per day [26]; and the final study also prescribed a 25% energy restriction, with a goal of 1.8 g of protein per kilogram of body weight per day, but no restrictions on times of consumption [27].

The eighth study included used a modified ADF protocol, with participants consuming ~25% of their energy requirements on every other day with ad libitum feeding on non-fasting days [28]. Participants were instructed to consume all their meals between 12 p.m. to 2 p.m. on fasting days. The comparison groups maintained their usual dietary habits.

3.4. Intervention—Resistance Training Protocol

The majority of studies [24–30] utilised a body building style workout, with 3–4 sessions per week, upper and lower or full body routines, varying from 3–15 repetitions per set and some working to failure [24,25]. Most of these studies performed the resistance training sessions in a fed state [24–28], with one study instructing participants to undertake their training session during their Ramadan fasting period [30], whereas a similar study compared training sessions performed during Ramadan fasting period and during the break in fasting (fed state) [29]. One study used a combination of resistance and aerobic exercise [28]. Participants completed 40 min of resistance training three times per week, varying between 10 to 15 repetitions utilising a combination of upper and lower body exercises. This was followed by at least 20 min of moderate intensity aerobic exercise (60–85% of maximal heart rate) on a motorised treadmill. Finally, in one study participants undertook two to five sessions per week at a 'weight training gymnasium', no other details were provided [31].

3.5. Effects of TRF on LBM, Body Weight and Fat

Moro and colleagues [24] reported no significant changes in LBM over the 8 weeks following TRF with an 8 h feeding window or control diet. However, the TRF group significantly reduced their fat mass (−1.6 kg ± 1.53, $p = 0.0005$) compared to the control diet. Similarly, Tinsley et al. [26] utilised an 8 h window for participants to consume their meals, but aimed for an overall small energy deficit (~250 kcal) in all groups. Despite a prescribed overall calorie deficit for both TRF and control diets, all groups combined significantly increased their body weight and LBM, with no difference between groups. Only the TRF plus HMB group experienced a significant reduction in body fat and body fat percentage compared to baseline, and only in the per protocol analysis, with intention to treat analysis showing no significant difference. While the TRF group did experience a mean reduction in these measures, this was not significant at week 8. However, all groups combined did experience a reduction in body fat percentage from baseline.

In a previous study from the same author, Tinsley et al. [25] used a shorter TRF protocol of 4 h and reported no significant changes in body composition in either the TRF or habitual diet (control) group. It should be noted that the authors did report a non-significant increase in average LBM (2.3 kg) in the habitual diet group, albeit with a small effect size (Cohen's d = 0.25). More recently, Stratton et al. [27] investigated the effects of TRF with an 8 h feeding window compared to a control diet, with participants in both groups prescribed a 25% energy deficit and 1.8 g of protein per kilogram of bodyweight per day. Both groups lost significant amounts of body weight, fat mass and body fat percentage, but experienced no change in LBM, with no differences between groups.

Table 2.1. Intermittent fasting combined with exercise and effect on body composition.

Author, Year	**Participant baseline characteristics**	**Drop outs (final number of completers)**	**Duration of study**	**Description of fasting**	**Description of exercise**	**Weight change**	**Body composition changes**	**Method of anthropometric assessment**	**Quality score (Downs and Black)**	**Fasted during exercise?**
Time restricted feeding studies										
Moro et al. (2016) [24]	n=34 Male Resistance trained (at least 3-5 times/week for 5 years) **Time restricted feeding group** n=17 29.9±4.1 years old BMI 26.5 kg/m²* BF% 13.0%* **Control diet group** n=17 28.5±3.5 years old BMI 27.2 kg/m²* BF% 13.2%*	NR – assumed full completion	8 weeks	**Time restricted feeding** 100% of energy needs consumed over 3 meals in an 8 hour window (1 pm, 4 pm and 8 pm), 20 g of whey protein after training **Control** 100% of energy needs consumed over 3 meals across the day (8 am, 1 pm and 8 pm), 20 g of whey protein after training	**Both groups** 3 resistance sessions/week, split routine, 6-8 repetitions at 85-90% of 1RM to failure, supervised sessions, conducted between 4pm-6pm	**Time restricted feeding** ↓1.0 kg **Control** ↑0.2kg	**Time restricted feeding** LBM ↑0.6kg (NS) FM ↓1.6kg **Control** LBM ↑0.5kg (NS) FM ↓0.3kg (NS)	DXA	18	No

Tinsley et al. (2017) [25]	n=28 Male Both groups recreationally active; have not followed a consistent RT programme over previous 3 months **Time restricted feeding group** n=14 22.9±4.0 years old BMI 27.2 kg/m²* BF% 21.3±5.4% **Normal diet group** n=14 22.0±2.4 years old BMI 24.3 kg/m²* BF% 18.7±3.8%	**Time restricted feeding group** 1 (13 completers) **Normal diet group** 5 (9 completers)	8 weeks	**Time restricted feeding** 4 days/week (non-training days) all energy consumed in a 4 hour window between 4 pm and midnight 3 days/week ad libitum **Normal diet** Usual dietary patterns	**Both groups** 3 resistance sessions/week on non-consecutive days, alternating upper and lower body, 8-12 reps to failure, 4 sets of each exercise	**Time restricted feeding** ↓1.0 kg (NS) **Normal diet** ↑3.0kg (NS) Analysis based on n=10 for time restricted feeders and n=8 normal diet due to exclusion for low compliance	**Time restricted feeding** LBM ↓0.2kg (NS) FM ↓0.6kg (NS) **Normal diet** LBM ↑2.3kg (NS) FM ↑0.8kg (NS) Analysis based on n=10 for time restricted feeders and n=8 normal diet due to exclusion for low compliance	DXA	16	No
Tinsley et al. (2019) [26]	N=40 Female Resistance trained (2 to 4 sessions per week for at least 1 year); BF% less than 33%	**Time restricted feeding group** 1 (12 completers) **Time restricted**	8 weeks	**Time restricted feeding** All energy consumed between 12pm and 8pm each day, prescribed energy deficit of 250 kcal	**All groups** 3 resistance sessions/week on non-consecutive days, alternating 2 different upper and lower body sessions	**Time restricted feeding group** ↑0.6kg **Time restricted feeding**	**Time restricted feeding group** FM ↓0.4kg LBM ↑0.9kg BF% ↓0.8%	4C	20	No

	Time restricted feeding group n=13 23.3±1.5 years old BMI 23.8 kg/m²* BF% 28.4±1.5% **Time restricted feeding group plus HMB** n=13 22.3±3.4 years old BMI 22.9 kg/m²* BF% 28.7±1.5% **Control diet group:** n=14 22.6±2.7 years old BMI 22.5 kg/m²* BF% 29.3±1.5%	**feeding group plus HMB** 3 (10 completers) **Control diet group** 5 (9 completers)		and protein of ≥1.4 g/kg/d. Instructed to consume whey protein supplement each day to achieve protein target **Time restricted feeding plus HMB** Same as time restricted feeding group, plus 3g/day HMB **Control diet group** Energy and protein targets as per the time restricted feeding group, however instructed to consume breakfast upon waking, and continue eating at self-selected intervals	Conducted between 12pm and 6pm	**group plus HMB** ↑0.6kg **Control diet group** ↑1.1kg Data from ITT analysis. Results significant for all groups combined.	**Time restricted feeding group plus HMB** FM ↓0.7 kg LBM ↑1.2kg BF% ↓1.4% **Control diet group** FM ↑0.4kg LBM ↑0.9kg BF% ↑0.1% Data from ITT analysis. Results significant for all groups combined.			
Stratton et al. (2020) [27]	n=32 Male Recreationally trained (2-4 sessions per week for at least 6 months)	**Time restricted feeding group** 0 (16 completers	4 weeks	**Time restricted feeding** All energy consumed between 12pm and 8pm, or 1m and	**Both Groups** 3 full body resistance sessions/week. Conducted between 3pm and 8pm.	**Time restricted feeding** ↓ 1.2kg **Control diet group**	**Time restricted feeding** FM ↓ 1.5kg LBM NS change (actual values NR)	4C	19	No

	Time restricted feeding group n=13 22.9±3.6 years old BMI 25.9 kg/m²* BF% 19.9±8.3% **Control diet group:** n=13 22.5±2.2 years old BMI 26.4 kg/m²* BF% 18.9±7.4%	**Control diet group** 2 (14 completers)		9pm each day, prescribed 25% energy deficit and protein intake of 1.8 g/kg/d. Provided 50g whey protein supplement on training days. **Control diet group** Energy and protein targets as per the time restricted feeding group, but with no time restrictions on consumption. Also provided with 50g whey protein supplement on training days.		↓ 1.4kg	BF% ↓ 1.6% **Control diet group** FM ↓ 1.4kg LBM NS change (actual values NR) BF% ↓ 1.5% Analysis based on n=13 for time restricted feeders and n=13 for control diet group due to exclusion for low compliance.			
Modified Alternate day fasting study										

Oh et al. (2018) [28]	n=45 Training history unclear, but described as 'unfamiliar with resistance execises' **Alternate day fasting + exercise group** n=12 male=5, female=7 37.3±7.3 years old BMI 27.5±2.6 kg/m² BF% 34.2±6.1% **Alternate day fasting group** n=13 male=3, female=10 32.9±7.3 years old BMI 27.6±2.8 kg/m² BF% 34.9±4.6% **Exercise only group** n=10 male=7, female=3 35.7±7.9 years old BMI 28.3±4.1 kg/m²	**Alternate day fasting + exercise group** 2 (10 completers) **Alternate day fasting group** 4 (9 completers) **Exercise only group** 2 (8 completers) **Normal diet group** 2 (8 completers) Sex of drop outs NR	8 weeks	**Alternate day fasting groups** 75% Calorie restriction alternating with ad libitum feeding **Normal diet/exercise only groups** No instruction given	**Alternate day fasting + exercise and normal diet + exercise groups** 3 training sessions per week consisting of 40 minutes of resistance training followed by 20 minutes of aerobic exercise on a treadmill. Resistance training was upper and lower body, 3 different sessions each week. Intensity ranged from 70% 10RM (15 repetitions) to 100% 10RM (10 repetitions) and altered each week. Aerobic exercise performed at 60-85% age predicted maximal heart rate	**Alternate day fasting + exercise group** ↓3.3±2.4kg **Alternate day fasting group** ↓2.4±3.1kg **Exercise only group** NS change **Normal diet group** NS change Data from ITT analysis	**Alternate day fasting + exercise** FM↓2.7±2.0kg LBM↓0.4±0 5kg BF%↓2.5±2.2% **Alternate day fasting group** FM ↓1.6±2 3kg (NS) LBM ↓0.5±0.9kg (NS) BF%↓1.3±2.4% **Exercise only group** FM↓1.2±1.9kg LBM↓0.1±0.9kg (NS) BF%↓1.1±1.8% (NS) **Normal diet group** FM↓0.3±1.3kg (NS) LBM↓0.2±0.7kg (NS) BF%↓0.1±1.5% (NS)	BIA	18	No

	BF% 31.0±5.0% **Normal diet group** n=10 male=4, female=6 40.6±10.0 years old BMI 26.3±3.0 kg/m² BF% 32.2±4.4%						Data from ITT analysis			
Ramadan fasting studies										
Trabelsi et al. (2013) [29]	n=16 Male Resistance trained (3 times/week for 1.6±0.6 and 1.5±0.5 years) **Fasted exercise n=8** 25.0±3.0 years old BMI 25.8 ± 4.0 kg/m² BF% 15.0±2.0% **Fed exercise n=8** 25.0±2.0 years old BMI 26.0±1.7 kg/m² BF% 14.0±1.0%	NR	4 weeks	**Ramadan fasting** Average fast 15 hours	**Both groups** 4 resistance sessions/week, 4-6 exercises, 4 sets at 10RM, split routine, supervised **Fasted exercise group** Exercise conducted between 4-6 pm before breaking fast **Fed exercise group** Exercise conducted between 9-10 pm	NS change in either group	**Fasted exercise group** LBM ↓0.2kg (NS) BF% ↓0.7% (NS) **Fed Exercise group** LBM ↑0.3kg (NS) BF% ↓0.4% (NS)	Skinfolds	17	Mixed – exercise conducted between 4-6pm for fasted group, unclear when fast began

Trabelsi et al. (2012) [30]	n=16 Male Recreational bodybuilders (at least 1 year experience) **Fasters n=9 (it is unclear whether this data is duplicated from Trabelsi et al 2013)** 24.0±3.0 years old BMI 26.0±0.7 kg/m^2	NR	4 weeks	**Ramadan fasting** Average fast 15 hours **Control group** Normal diet	**Both groups** 4 resistance sessions/week, 4-6 exercises, 4 sets at 10RM, split routine, supervised	**Fasters** NS change **Non Fasters** ↑1.9 kg	**Fasters** FM ↓0.6kg (NS) LBM ↓0.1kg (NS) BF% ↓0.7% (NS) **Non Fasters** FM ↑1.2kg (NS) LBM ↑0.7kg (NS) BF% ↑1.1% (NS)	Skinfolds	16	Yes – exercise conducted between 4-6pm, unclear when fast began

	BF% 14.5±2.0% **Non fasters n=7** 26.0±3.0 years old BMI 26.0±1.5 kg/m² BF% 13.5±1.4%									
Stannard et al. (2008) [31]	n=8 Male Recreationally active (2-5 sessions/week) 24.1±0.8 years old BMI 24 kg/m²* BF% 12.9±3.5%	NR	4 weeks	**Ramadan fasting** Average fast 14.5 hours	2-5 sessions/week in the weight-training gymnasium. Type and duration of exercise is unspecified	↓1.3kg	FM ↓0.7 kg LBM ↑0.1kg (NS) BF% ↓0.7% (NS)	Underwater weighing	16	Unclear

Notes: All results presented as mean ± SD. Where no SD has been reported in the original study, values are means. Baseline characteristics are representative of all participants before drop outs. *Data not reported, calculated by the authors from baseline height and weight (BMI), or from baseline weight and fat mass for BF%. 1RM = 1 repetition maximum, 10RM = 10 repetition maximum, 4C = 4 compartment model, BF% = body fat percentage, BMI = body mass index, DXA = dual x-ray absorptiometry, FM = fat mass, HMB = β-hydroxy β-methylbutyrate, ITT = intention to treat, LBM = lean body mass, NS = non significant change, NR = not reported, PP = per protocol, RT = resistance training.

3.6. Effect of Modified ADF on LBM, body weight and fat

In the one study that investigated modified ADF [28], the authors separated participants into 4 groups: modified alternate day fasting plus exercise group, modified alternate day fasting only, exercise plus habitual diet and habitual diet only. Intention to treat analysis revealed a significant decrease in LBM (-0.4 ± 0.5 kg) in the modified ADF plus exercise group from baseline ($p<0.01$), though this was not statistically different from the habitual diet only group. All other groups reported no significant change in LBM compared to baseline. Both modified ADF with exercise and modified ADF alone reported significant decreases in body weight (-3.3 ± 2.4 kg and -2.4 ± 3.1 kg respectively) compared to baseline, though this was only significant compared to the control diet in the combination group. The combined group also reported significant decreases in both fat mass and body fat percentage, -2.7 ± 2.0 kg and -2.5 ± 2.2%, respectively compared to baseline and this was significantly different to the habitual diet only group. However, the modified ADF only group reported a significant decrease in body fat percentage only (-1.26 ± 2.4%) compared to baseline.

3.7. Effect of Ramadan fasting on LBM, body weight and fat

Trabelsi and colleagues published 2 studies investigating Ramadan fasting in conjunction with resistance training 4 times per week in recreational bodybuilders [29, 30]. In both studies, the fasting groups demonstrated no significant change in LBM, body weight, body fat mass, and body fat percentage [29, 30]. In the 2012 study [30], Trabelsi incorporated a non-fasting habitual diet control group. Compared to the Ramadan fasters, the control group demonstrated a significant increase in body weight (~1.9 kg, $p<0.05$), with non-significant increases in both fat mass and LBM. In the 2013 study [29] a strength of the study design was that authors controlled for diet prior to exercise, in order to determine if exercising in a 'fasted' versus ‘non-fasted, fed’ state influenced changes in body composition. Similar changes in body composition were observed for both fasted and fed exercise groups.

The study by Stannard et al. [31] found that recreationally active males undertaking 2-5 sessions of ‘weight-training gymnasium’ exercise in conjunction with Ramadan fasting resulted in no significant changes in LBM, despite an average body weight loss of -1.3 kg (p=0.019) and a -0.7 kg reduction in fat mass (p=0.033).

3.8. Methods of anthropometrical assessment

The anthropometric assessment used in the studies varied with skinfold tests [29, 30], bioelectrical impedance analysis (BIA) [28], dual x-ray absorptiometry (DXA) [24, 25], underwater weighing [31], and a 4 compartment model, combining DXA and bioimpedance spectroscopy [26] or DXA, bioimpedance spectroscopy and air displacement plethysmography [27] used.

3.9. Reported adherence to dietary protocols

Only 2 of the included studies reported adherence rates to the fasting protocol [25, 26]. Adherence rates were calculated by dividing the number of days adherent by the total number of fasting days [25, 26]. Tinsley et al. [25] reported a high level of adherence, with participants completing an average of 95.9 ± 4.1% of the 32 TRF fasting days over the 8 week duration. However, 3 of the 14 participants (21%) from the TRF intervention group were excluded from the overall analysis due to a <80% compliance, while another participant dropped out due to unknown reasons. Within the control group (habitual diet), 6 of the 14 participants (43%) did not finish the intervention. Participants also rated the difficulty of adhering to the TRF intervention using a 10 cm visual analogue scale, with a rating of zero on the left hand side of the scale indicating program adherence was 'extremely easy', and 10 on the right hand side indicating adherence was 'extremely difficult'. After 4 weeks, difficulty was rated at 3.6 ± 1.4 out of 10, and did not change significantly by the end of the study (3.8 ± 2.2, p=0.86). Some participants reported difficulty initially with the fast regime, but commented that it became easier after several days. In their other study, Tinsley et al. [26] reported a 89 ± 8% compliance with their meal timing protocol based on intention to treat analysis, increasing to 91 ± 3% using per protocol analysis. Participants were excluded from the per protocol analysis if they had less than 80% adherence to the eating schedule, or completed less than 22 of 24 resistance training sessions. Overall, 12 of 13 participants allocated to the TRF and 10 of 13 in the TRF plus HMB group completed the study, while only 9 of 14 completed in the control group. In other studies, adherence was monitored by participants completing dietary logs every day during the intervention period [28] or through a structured interview performed by a dietitian [24]. However, dietary data and adherence was not reported. None of the Ramadan fasting studies included measured adherence to the protocol.

3.10. Quality Appraisal

All studies included in the present review scored between 16-20 points on the Downs and Black checklist out of a possible 27 [24-31].

4. Discussion

To the authors' knowledge, this is the first review to systematically analyse the published literature on the combined effects of IF and resistance training on changes in LBM in recreationally active and non-elite trained individuals. The major finding is that despite heterogeneity in study design, especially fasting method, LBM was generally maintained when IF and resistance training were undertaken concurrently during the short to medium term. Though 4 studies observed mean increases in LBM [24, 26, 29, 31], only one study reported a statistically significant increase (0.9 kg - 1.2 kg) [26]. Interestingly, the study that reported the statistically significant increase in LBM also observed a mean increase in participants' body weights, which is in contrast to all other studies and indicates energy balance may be a potential contributing factor. Thus, it remains unclear whether LBM accrual is possible when fasting combined with resistance training results in an overall energy deficit. Additionally, the length of the intervention may also be a factor given most studies were only 4-8 weeks in duration. Therefore, further research is needed to examine the longer term effects of these interventions, but also other forms of IF, with the majority of studies focusing on TRF and Ramadan style fasting.

For many individuals, the primary goal of resistance training is to increase LBM, or at the very least, maintain it. Of the 8 studies included and reviewed, only 1 study showed a reduction in LBM from baseline when IF and resistance training were undertaken together [28]. However, it should be noted that this reduction was small, and although significant, was not significantly different to the other experimental groups in the study [28]. This small reduction could be due to the addition of aerobic exercise (20 minutes) at the end of each resistance training session with evidence suggesting that concurrent training (performing both resistance and aerobic exercise together) may compromise resistance training-induced muscle adaptations due to the "concurrent training effect" (CTE) [32]. However, whether CTE is the reason for the LBM reduction in the Oh et al. [28] study is unknown.

Whilst the majority of studies included suggest LBM can be maintained when undertaking IF and resistance training together, whether or not the accrual of LBM is compromised is less clear. A number of studies did report mean increases in LBM [24, 26, 29, 31], however, only 1 study reported a statistically significant increase (0.9 – 1.2 kg) [26]. This was evident by a main effect for time, with no difference (interaction) between the TRF or normal diet groups. Of particular note is that despite participants in this study being prescribed an energy deficit of ~250 kcal per day, energy intake actually increased over the intervention period which led to an increase in mean weight for all groups by the end of the study. Given this was the only study to show a meaningful and significant increase in LBM, it does suggest that an apparent energy surplus (as evidenced by a mean increase in weight) could be a major determinant in supporting training-induced adaptations, such as muscle growth [33]. Therefore, it raises an important question of whether IF compromises growth of LBM when undertaking resistance training without an apparent energy surplus. Interestingly, changes in LBM were not statistically different between intervention and 'normal diet' control groups in the studies that included such a comparative group [24-28, 30]. This is somewhat surprising given most, though not all, resistance training interventions lead to an increase in LBM [34]. It is possible the length of training (i.e. 4-8 weeks) used in the reviewed studies did not allow adequate time for significant growth to occur. Although the time-course of muscle hypertrophy is poorly defined, it is usually expected to begin within a couple of months for untrained individuals, with those with prior training experience observing the effects later [35]. Furthermore, while most training programs were of similar frequency across studies (i.e. 3-4 sessions per week), number of repetitions, load and whether or not participants worked to failure varied, which may have affected total volume of work. Indeed, variation in overall volume, as opposed to frequency seems to have greater impact on muscle growth [36]. Thus, it is likely that the relatively short durations and variations in volume/effort of training programs used in the reviewed studies, rather than IF per se was an important determining factor in the observed effects on LBM. It is clear that further research is needed that incorporates longer training programs (>8 weeks) to gain a better understanding of the impact of IF on training-induced adaptations.

Protein intake is another major determinant of training-induced changes in LBM [37]. Of the 6 studies that reported it [24-27, 29, 30], protein intake ranged from 1.2 g/kg/day to 1.9 g/kg/day, which is close to or above the level recommended for LBM maintenance and accrual [37]. In the one study that reported a LBM loss [28], no protein intake was reported, and indeed no emphasis appeared to be given to protein intake for participants. While it is possible that insufficient protein intake could be a contributing factor for the observed LBM loss, participants in this study also lost the most weight compared to all other included studies, possibly due to a combination of the more severe energy restriction protocol used and the extra aerobic exercise component included. Thus, larger weight reductions and periods of greater energy deficit (though not directly measured) could also be a contributing factor.

The observed effects of IF and resistance training on fat mass and body fat percentage were less consistent. Five studies reported statistically significant changes in body fat mass or body fat percentage [24, 26-28, 31]. The remaining 3 studies [22, 26, 27] reported no significant changes. Body fat losses ranged from an average of 0.7 kg to 2.7 kg, while body fat percentage reductions ranged from 0.8-2.5%. The variability in fat loss is likely a reflection of intervention duration, total energy intake and participant characteristics such as sex and baseline weight status. The modified ADF study [28] demonstrated the greatest weight and fat loss compared to other fasting protocols. This is to be expected given they prescribed the most severe energy restriction, included an extra aerobic exercise component and had the equal longest duration. However, weight loss does not appear to be a pre-requisite for changes in body fat or body fat percentage in the studies reviewed. While 4 of the 5 studies that showed changes in these measures resulted in (generally small) concomitant weight loss [24, 27, 28, 31], 1 study showed a reduction in fat despite a gain in weight [26]. This provides evidence that IF with resistance training can improve body composition (at least in regards to body fat loss) independent of large energy deficits. Changes in metabolic fuel preference during fasting is a likely mechanism behind the enhanced fat loss, with a decline in carbohydrate oxidation and increases in fat oxidation, ketogenesis and gluconeogenesis evident [38, 39]. However, whether these contribute to meaningful changes in body fat levels requires further investigation.

There are several limitations associated with this review and the included studies. Firstly, our review only considered changes in whole body LBM, which does not capture local changes in muscle hypertrophy. Half of the included studies (all TRF studies) did use more sensitive assessments of muscle hypertrophy (i.e. muscle ultrasound). While 2 studies showed similar directional change in comparison to the whole body LBM changes assessed by DXA or a 4C model [24, 27], the other 2 reported conflicting results [26, 27]. Given the majority of studies comparing these different techniques have been undertaken in clinical (i.e. sarcopenic) populations, it is clear that further work is needed in populations included in this review. Secondly, most studies did not include an IF only comparator group, making it unclear whether there is an additive effect of resistance training on LBM compared to IF alone. Thirdly, the included studies were all of a short to medium duration, and results cannot be generalised to longer intervention periods. Finally, while the authors tried make our search comprehensive, we focused on published literature only.

5. Conclusions

Lean body mass is generally maintained when IF, including when followed for religious reasons, is combined with resistance training. However, whether or not IF inhibits LBM accrual is unclear and may be contingent on adequate provision of protein and energy balance. The combination of IF and resistance training may also lead to a reduction in body fat, not only during apparent energy deficit but also energy surplus. Given low muscle mass and poor muscle strength are important risk factors for disability and potentially mortality, especially in older individuals, these findings may also have important clinical implications. Future research should aim to examine the longer-term effects of various IF regimes with resistance training on LBM, incorporating varying levels of energy intake, and where possible, appropriate non-exercise and non-fasting control groups. Furthermore, given the growing popularity of IF for weight loss, future research should also consider whether IF paired with resistance training is more or less effective and sustainable compared to other traditional forms of energy restriction diets.

Author Contributions: Conceptualization, S.K and R.B.; methodology, S.K.; writing—original draft preparation, S.K.; re-writing—S.K., M.C.; review and editing, S.K., M.C and R.B.; supervision, M.C and R.B.. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors have no relevant sources of funding to disclose.

Conflicts of Interest: The authors declare no conflict of interest.

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2.3 Potential mechanisms of LBM protection and fat mass loss

While the previous section detailed the current state of the literature on how IF affects LBM when combined with resistance training, it did not discuss in detail the mechanisms that may help promote LBM retention or growth while also enhancing fat loss.

Firstly, in terms of body fat reduction, prolonged periods of fasting alter fuel oxidation, such that the body preferentially utilises fatty acids instead of glucose, increasing fat oxidation and loss (24). A number of trials have shown that individuals undertaking ADF, TRF or Ramadan experience increased levels of fat oxidation (25–28). This may partly explain why some studies report significant fat loss with IF without the need for significant reductions in energy intake. For example, using a cross-over design, Stote et al. (26) found that participants experienced a reduction in body weight and fat, and a trend towards an increase in LBM when consuming 1 compared to 3 meals per day, despite minimal reported changes in dietary intake (~65 kcal/day). While these results are intriguing, studies have shown that substrate utilisation is not significantly different in individuals losing weight via IF and CER (29), and the reduction in weight without a reduction in energy intake shown in the Stote et al. study (26) may instead reflect inaccurate dietary reporting or non-compliance. Alongside changes in substrate utilisation, some studies (30,31), though not all (32,33), have shown that IF can lead to attenuation of the BMR reductions commonly seen during weight loss, though there has been no consistent benefit or detriment found when compared to CER. Notwithstanding, any attenuation in this adaptive response to energy restriction may help improve rate and maintenance of weight and fat loss at relatively higher energy intakes compared to if BMR reductions occurred. While a clear mechanism for any potential benefit of IF is not yet apparent, studies in humans and rodent models have illuminated some intriguing effects of

IF on energy expenditure. In mice, IF promotes browning of white adipose tissue (34,35), which is associated with increased energy expenditure through non-shivering thermogenesis. In both humans and mice, brown adipose tissue is also related to increased diet induced thermogenesis (36,37). Given its ability to prevent obesity in rodent models, brown adipose tissue has recently received increased attention as a potential therapeutic target for the treatment of overweight and obesity in humans (38). Whether or not IF leads to increased levels of brown adipose tissue in humans, and its implications for BMR and weight are unclear, however this may provide an interesting avenue for future IF research.

Secondly, in terms of LBM maintenance, fasting causes a number of metabolic and hormonal shifts that may support this. Nutrient deprivation leads to increased levels of skeletal muscle Sirtuin 1 (Sirt1) (24), an enzyme that has recently been shown to enhance muscular hypertrophy in mice via a number of cell-dependent pro-synthetic and anti-catabolic pathways (39). Sirt1 also promotes mitochondrial biogenesis, resulting in greater fat oxidation in order to maintain energy homeostasis during fasting (which may further assist with reductions in body fat discussed above) (40). When fatty acids become a major fuel source, ketone production is increased, which may enhance the preservation of LBM. The production of ketones may partially act to preserve muscle via a reduced need for amino acid precursors for gluconeogenesis as the body shifts away from glucose as a fuel source (41). Indeed it has been shown that infused beta-hydroxybutyrate, the most abundant circulating ketone body, inhibits leucine oxidation and promotes protein synthesis in humans (41). Concurrently, hormonal changes seen during fasting may also assist with LBM-preservation. Low blood glucose, as seen in fasting individuals (42), is a potent stimulus of adrenaline secretion (43), a hormone which has been shown to stimulate myogenesis in humans via a number of protein kinase A and protein kinase A independent pathways (44). However, fasting individuals also experience a sustained increase in adrenaline preceding any reduction in blood glucose, thus suggesting lowered blood glucose may not be a pre-requisite (42). This adrenergic response may be further heightened by exercising in a fasted state (45). Although fasted exercise may also cause a higher muscle protein breakdown in the hours following exercise (46), this may be rescued by immediate post-exercise nutrition provision (47). While it could therefore be speculated that fasting, followed by subsequent exercise and immediate provision of nutrition during the recovery period may provide an optimal hormonal environment for LBM-preservation and growth, this is yet to be adequately explored. Furthermore, exercise in the fasted compared to fed state may also reduce overall intensity and performance in resistance (48) and intense aerobic activity (49), which could nullify the potential benefits of fasting. Indeed IF has the unique ability of allowing individuals to complete exercise in an acutely more well-nourished state than CER, while potentially benefiting from the increased fat oxidation, and ketone and hormone-associated LBM preservation on fasting days as discussed earlier. However, as alluded to in the previous section, more research needs to be conducted to see whether or not there are tangible benefits of IF over more traditional restriction methods.

2.4 The effects of intermittent fasting on cardio-metabolic health markers

While it is well known that the excess adiposity associated with being overweight/obese increases the risk of a number of diseases, including type 2 diabetes and cardiovascular disease (50), this relationship is also seen in normal weight individuals with excess levels of body fat (51). The increase in risk is likely driven by a number of cardio-metabolic derangements associated with excess body fat including insulin resistance (increased fasting levels of glucose/insulin) (52,53), dyslipidaemia [excessive total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C); low levels of high-density lipoprotein-cholesterol (HDL-C); and high triglycerides] (54) and chronic inflammation (55). As discussed previously in this chapter, the primary goal of energy restricted diets is to reduce weight and body fat, and therefore reduce the disease risk factors associated with excess body fat. Indeed even losing small amounts of weight has been shown to significantly reduce the risk of developing type 2 diabetes in individuals with impaired glucose tolerance (56).

As discussed, weight loss is traditionally induced utilising CER alone or combined with exercise. However, the possibility that restricting energy via IF may have unique impacts on cardio-metabolic health has been mooted, and could be potentially due to a process termed 'flipping the metabolic switch' (24). Flipping the metabolic switch refers to the point at which endogenous liver glycogen stores are depleted (typically 12-36 hours after ceasing food consumption), and the body 'switches' to the use of free fatty acids as a predominant source of energy (24). This is also one of the mechanisms proposed in section 2.3 to be protective of LBM during periods of fat loss. During fasting, the depletion of hepatic glycogen stores leads to increased mobilisation of lipids from adipose tissue as free fatty acids, which are metabolised via β-oxidation into ketone bodies (β-hydroxybutyrate and acetoacetate) and utilised to generate ATP (24). This is likely an evolutionarily conserved mechanism for survival during periods of starvation, allowing the utilisation of man's vast ability to store a large amount of lipids (57). Frequent periods of heightened fatty acid oxidation seen in fasting may promote a redistribution of fat towards subcutaneous sites and away from visceral sites (58). As accumulation in the latter has been implicated in insulin resistance and dyslipidaemia (59), this provides a potential mechanism for improving cardio-metabolic health via IF that would not occur during CER.

Research in rodents generally shows reasonably consistent benefits for glucose (60–66) and lipid metabolism (67,68) as a result of IF. Similarly, results from human studies appear to show that IF is generally effective at improving blood lipid profiles and in some cases markers of glucose metabolism, although efficacy seems to vary depending on the type of IF.

2.4.1 Alternate day fasting studies

Studies utilising ADF show that this form of fasting leads to improvements in blood lipids, including reductions in TC, LDL-C or triglycerides (69–77). These changes generally appear to be similar in magnitude to CER when these two diets are compared (74,75,78). However, Hutchison et al. (69) showed that ADF resulted in greater reductions in TC and LDL-C than CER even after adjusting for slightly greater weight loss in the ADF group. They also showed mean improvements in TC, LDL-C and triglycerides that were similar to the CER group in an ADF group designed to maintain weight (via consumption of 145% of energy needs on non-fasting days), despite significantly less weight loss. This raises the possibility that ADF may benefit blood lipid profile independent of weight loss. Similarly, ADF has generally led to reductions in fasting glucose (75,79,80), in some cases over and above CER (though this may have been mediated by greater weight loss) (80); and insulin (75,79,81).

2.4.2 Time-restricted feeding studies

TRF appears to have less consistent effects on cardiometabolic health markers than ADF. This might be expected given the majority of studies utilising this form of IF haven't induced substantial amounts of weight loss. Nonetheless, as discussed above, some forms of IF do result in beneficial changes that appear to be independent of weight loss. TRF generally does not appear to reduce TC or LDL-C (25,82,83) and may in fact increase these measures in some instances (26,84). In the latter of these two studies, Jamshed et al. (84) used a cross-over study design to investigate the effects of early time restricted feeding (all food consumed between 8 am and 2 pm) compared to meals spread evenly across the day (meals consumed between 8 am and 8 pm). This led to a fast that was 6 hours longer in the lead up to blood samples being taken when following the TRF regimen, which the authors supposed could have been responsible for the increase in TC and LDL-C due to a greater reliance on fat oxidation. While previous research seems to indicate that length of fasting (up to 16 hours) has a minor impact on blood cholesterol levels (85), the variations shown in the Jamshed et al. study do seem to fit within the estimated variability of <10%. Triglycerides have been shown to both decrease (25,26,82), remain unchanged (84) and increase (83) in response to TRF. The latter study (83) was also an early TRF study with a similar protocol to Jamshed et al. (84) and these authors also postulated that increased triglycerides may have been caused by a longer fast prior to sampling. While triglycerides appear to be more greatly affected by fasting duration (85), this does not explain why a similar response was not seen in participants in the Jamshed et al. (84) study, where triglycerides were unaffected. Responses in glucose metabolism also appear to vary, with studies showing increases in glucose (86), decreases in both glucose and insulin (25), glucose only (87), insulin only (82,83) or no difference (26,88). The variance seen in TRF may be a result of differing windows of consumption (this ranges from 4 to 10 hours) or the time of day which these windows span. Early (83,87) or mid-day (82) consumption windows appeared to more consistently result in better outcomes in terms of glucose metabolism. This may be due to natural hormonal shifts driven by circadian rhythm, with melatonin, a hormone that rises in the lead up to habitual bedtime (89), attenuating glucose-stimulated insulin release and impairing glucose metabolism later in the day (90).

2.4.3 5:2 intermittent fasting studies

Studies investigating 5:2 IF have been shown to cause reductions in TC, LDL-C and triglycerides (29,91–95), with values generally similar to CER in those that have included comparator groups (92–95), though one study showed greater reduction in post-prandial triglyceride response in their 5:2 fasting group (29). Interestingly, some research has shown that 5:2 IF may improve glucose metabolism to a greater degree than CER in some instances. In two separate studies (93,95), Harvie et al. showed that 5:2 IF resulted in greater reductions in fasting insulin and in the homeostatic model assessment of insulin resistance (HOMA-IR) when compared to CER. Interestingly, both of these studies utilised consecutive fasting days, whereas others have generally prescribed fasting on non-consecutive days, likely with the aim of enhancing tolerability of the diet. Templeman et al. (96) proposed that completing fasting on consecutive days may be a key requirement for inducing the metabolic benefits of IF as it would result in longer periods of continuous fasting. 5:2 IF usually includes some form of consumption on fasting days (as does modified ADF) that breaks the fast and may undermine its metabolic benefits. As discussed earlier, the switching of metabolic fuel from glucose to lipids as a predominant source may be responsible for the proposed benefits of IF (24). Consecutive fasting days when utilising 5:2 IF is much more likely to result in periods of fasting that extend into the 12-36 hour period requisite for the metabolic switch, and may therefore explain some of the disparities in cardio-metabolic benefits seen with this style of fasting. However, it should be noted that improvements in glucose metabolism have not been shown to be superior in 5:2 fasting in all studies that have used consecutive fasting days, with Antoni et al. (29) finding no difference in glucose tolerance compared to CER.

Overall, the weight of evidence would suggest that most forms of IF offer similar cardio-metabolic benefits to CER, though some studies have shown improvements in glucose or lipid metabolism to be superior, and others have shown benefits of IF independent of weight loss. Given the variation in IF protocols, this variability is not overly surprising. Furthermore, there appear to be no studies that have investigated the comparative effects on cardiometabolic markers of different styles of IF, with studies generally including either a CER group or no comparator group. Thus whether one style of IF offers superior outcomes to others is also unclear. Regardless, given one of the main purposes of restricting energy intake is to reduce weight, body fat and subsequently improve risk of disease, measurement of these cardiometabolic markers in any study implementing these dietary strategies is of importance.

2.5 The effects of intermittent fasting on compliance, mood and hunger

As mentioned in the introductory chapter, while energy restricted diets are effective at inducing weight and fat loss and improving health outcomes, efficacy is limited by dietary compliance. One of the major drivers behind the growing body of research into IF is a noted general poor compliance to traditional CER (7), and the possibility that if all energy restriction is concentrated into short time periods, compliance might be greater. Energy restricted diets are difficult, may lead to feelings of hunger, deprivation, reduced mood and pre-occupation with food (8), and can be further undermined by an individual’s social network (97). Continuous energy restricted diets, as the name implies, are continuous, and theoretically allow little flexibility. As they are continuous, they require constant restraint and any deviation from the diet on any day may be seen as a failure. IF aims to remedy this by generally allowing ad libitum or relatively greater intake on non-fasting days, thus meaning that individuals only need to practice such restraint for set periods of time. As noted for previous sections, the effects of each type of IF on compliance, and the factors that may underlie compliance (e.g. hunger, satiety, cravings and mood) likely vary.

2.5.1 Alternate day fasting studies

ADF studies have consistently reported high levels of compliance, ranging from 78% to 98% of completed fasting days (70,72,73,98,99), while another study reported a subject-rated compliance level of around 3.5/5 ‘stars’ in both ADF and CER groups (75). In contrast, however, a different ADF study, that also included a CER comparator group, showed that compared to alternate day fasters, continuous energy restrictors were more compliant with their energy goal at months 3, 6, 9 and 12 during their year-long intervention (78). This data suggests that it may not be 100% clear which diet is more beneficial for compliance, and other factors like hunger and mood may play an important role.

A number of ADF studies have included details on the effects of this type of fasting on hunger and mood. Heilbronn et al. (27) and Varady et al. (73) both noted moderate-high levels of hunger that didn’t change over the course of their studies (22 days and 12 weeks respectively), though in the Varady study there was an increase in satisfaction and fullness from baseline to post-intervention. Johnson et al. (77) showed in a group of asthma sufferers that while hunger did not increase over baseline, unsurprisingly it was higher on fasting than non-fasting days. Furthermore, it is logical to suppose that there might be a continuum for hunger that may increase depending on overall average restriction, time spent restricting and severity of restriction on fasting days. Indeed in a study that included a CER group and an ADF group that undertook an overall 30% energy restriction, and an ADF group that was prescribed a 0% energy restriction (via an increase in intake on non-fasted days), the CER group experienced the greatest level of hunger, followed by the 30% restricted ADF group, with the ADF 0%

restriction group reporting the lowest hunger levels (69). One study reported changes in mood (74), reporting an improvement in positive and a decrease in negative mood that was similar between ADF and CER groups.

2.5.2 Time-restricted feeding studies

TRF could be considered a less severe iteration of IF. Even though fasting periods are generally absolute with this version (i.e. water only fasting during the fasting periods), some form of intake occurs every day, the knowledge of which may aid in compliance. Indeed compliance appears to generally be greater in TRF than either ADF or 5:2 IF. While no studies to date have compared levels of compliance to these diets in the same study, compliance rates for TRF protocols have ranged from 84% to 100% (83,88,100–102). While similar rates have been reported for both ADF and 5:2 fasting, much lower compliance rates have also been reported, which isn't the case with TRF, indicating it may be more tolerable. However new research undertaking direct comparisons between fasting versions would elucidate this more clearly.

Only a handful of studies have reported on how TRF affects hunger or mood variables. Two cross-over studies investigating early TRF showed differing effects on levels of hunger in the evening. In the first study, Sutton et al. (83) showed that over 5 weeks, consumption of all energy over a 6 hour window, with dinner prior to 3 pm, resulted in: a reduced capacity and desire to eat; a trend towards a reduction in hunger and; an increase in fullness when compared to 5 weeks of a standard 8 am to 8 pm eating schedule. Conversely, Ravussin et al. (100) used a similar cross-over design and found that early TRF led to increased hunger and desire to eat, and reduced overall and stomach fullness. It should be noted that the latter of these 2 studies was only 4 days in length, thus it is possible that adaptation to this eating schedule may occur over longer periods of time. In another cross-over trial, Stote et al. (26)

found that over the course of 8 weeks, consuming all energy in one meal (between 5 pm and 9 pm) led to greater levels of hunger, desire to eat and prospective food consumption, and reduced fullness compared to consumption of 3 meals per day. In conjunction with the Sutton et al. study (83) this might suggest superiority of early TRF compared to later feeding windows for reducing hunger. However, in comparison to the Stote et al. (26) study, where participants completed their responses daily, those in the Sutton et al. (83) study completed their responses only once at the end of the study as a representation of the previous week, which may have introduced recall bias. Finally, Hutchison et al. (87) showed that there were no differences in hunger, fullness or desire to eat in response to a test meal after 7 days of either early TRF (consumption between 8 am and 5 pm), or day-time TRF (12 pm and 9 pm).

2.5.3 5:2 intermittent fasting studies

Studies in 5:2 IF have shown that compliance to dietary protocols are generally high. However, there is considerable variability, with some self-reported compliance to energy targets on fasting days as low as 20% after 6 weeks (103) and others as high as 98% after ~7 weeks (104). Other studies have generally reported compliance levels of 74% to 97% (29,93,94,105,106), although a number did report a reduction in compliance over time (93–95,105). It's unclear why such variability in compliance exists, although it should be noted that there are differences in the way 5:2 IF (as with all IF) is implemented. This is evident in differing allowances on fasting days in terms of energy, type of food (liquid versus solid), duration and whether fasting days are consecutive or not amongst others. However, there appears to be no clear pattern to this that might predict better or worse compliance based on currently published literature. Despite variability, most studies that included a CER comparator group showed similar levels of compliance between groups (94,95,105), with one exception (93). In this final study, Harvie et al. (93) reported that ~75% of fasting days were successfully completed after 3 months in their 5:2 IF groups, while those in the CER group only reached their energy target on 39% of days.

The effects of 5:2 IF on other factors such as mood and energy are less often reported. Harvie et al. (93) reported that hunger was greater in their fasting group than in a comparator CER group at the beginning of their study, but this difference was attenuated at months 3 and 4, suggesting adaptation in the fasting group. They also found that both groups experienced similar reductions in scores for tension, depression, anger, fatigue and total mood disturbance and an increase in vigour, with no differences between interventions. Similarly, Carter et al. (107) found increases in feelings of fullness and satisfaction across 12 weeks in their 5:2 IF and CER groups. Conversely, two other studies found that 5:2 fasters reported feeling hunger more often compared to continuous energy restrictors at 3, 6 and 12 months (105,108).

Overall, previous studies indicate that compliance to IF protocols is generally high, however can vary greatly, with TRF potentially superior to alternate day or 5:2 fasting (though this hasn't been directly compared). Furthermore, variability exists in how these diets affect measures of hunger, satiety and mood, which may be a function of real differences in diets, or of how and when these are measured. Regardless, future studies should include measures of these as they may provide important information on the practicality of IF as a means of successful weight loss and longer term maintenance.

2.5 Conclusion to chapter

This literature review has shown that while research into IF has increased substantially over the past 10 years, there remain a number of gaps and research questions still to be answered. There is an obvious lack of research comparing the effects of IF (with the exception of TRF) and CER on body composition when both diets are combined with resistance training. This is surprising given that one of the proposed advantages of IF over CER is the potential to improve outcomes in terms of LBM, and that resistance training is known to improve maintenance and growth of LBM during weight loss. Consequently, there is also little detail on how this combination might effect cardio-metabolic markers. Finally, a number of studies have reported similar compliance levels and generally similar results in terms of how IF and CER affect hunger and mood. Nonetheless, the possibility that IF might promote greater compliance is still a key driver of research into this area, and given the variety of ways in which IF can be implemented, should continue to be investigated and reported on.

Chapter Three

Chapter Three: Aims and objectives

Chapter 2 identified that there are a lack of studies investigating the combination of resistance training and IF, with the exception of TRF. This is surprising given one of the purported potential benefits of IF is better outcomes for LBM. To date no studies have explored the effects of 5:2 fasting with resistance training in comparison to CER. It is also important to explore how different combinations of diet and exercise, and fasting protocols affect compliance and cardio-metabolic risk factors, particularly in individuals with excess adiposity.

Thus, the aim of this project was to investigate the following research questions and hypotheses:

1. Are there differential effects on body composition, muscle size and quality or strength when undertaking a resistance training program combined with either 5:2 IF or CER when diets are matched for energy deficit and protein intake in individuals with excess body fat?

Hypothesis: 5:2 IF will result in greater maintenance of LBM and muscle growth than CER when both diets are combined with resistance training, but similar increases in strength.

2. Are the two interventions comparable in terms of their effect on blood markers of cardio-metabolic health?

Hypothesis: Both 5:2 IF and CER will result in similar improvements in markers of cardio-metabolic health when combined with resistance training.

3. Does either dietary intervention lead to different levels of compliance, or ratings of mood, hunger, cravings or energy levels?

Hypothesis: 5:2 IF will lead to higher average ratings of compliance, mood and energy levels, and reduced hunger and cravings compared to CER.

These research questions are addressed in the subsequent chapters reporting results from a 12 week randomised intervention study, in which participants undertook a 5:2 IF style or CER style diet, while completing 3 exercise sessions each week. The methodology and methods for this study is detailed in the following chapter.

Chapter Four

Chapter Four: Methodology and methods

This chapter describes the methodology and methods utilised to conduct a 12 week intervention investigating the research questions raised in Chapter 3. This chapter contains sections submitted for publication in peer reviewed journals. Further, as this chapter contains detailed methodology and methods for the entire project, the methods sections for the manuscripts in subsequent chapters have been removed to avoid repetition. However, each chapter still contains the statistical methodology for each manuscript presented, which has not been presented in this chapter.

4.1 Overall design

This project investigated the impacts of two energy-restricted dietary approaches combined with resistance training on measures of body composition, muscle size and quality, muscular strength and endurance, dietary intake, dietary compliance, measures of mood, hunger and satiety and blood markers of cardio-metabolic health in individuals who had not recently undertaken a structured resistance training program, and had excess levels of adiposity. Specifically, the 12- week project examined two dietary interventions:

1. 5:2 IF: participants were prescribed approximately 30% of energy requirements (in the form of high protein meal replacement shakes and soups and vegetables) on 2 non-consecutive days per week, with 5 days of eating 100% of energy requirements, including an average overall prescription of ≥1.4 grams of protein per kilogram of body weight per day (g/kg/day) and;

2. CER: participants were prescribed ~80% of their daily energy requirements each day of the week, including an average overall prescription of ≥1.4 g/kg/day of protein.

Participants in both dietary intervention groups undertook 2 days per week of ~45 minutes of supervised, intense resistance exercise (utilising mainly bodyweight exercises), and 1 day per week of ~ 30 minutes of unsupervised exercise combining aerobic and resistance training. The training was progressively overloaded over the 12 weeks. Measures of body composition, muscle size and quality, muscular strength and endurance and blood markers of cardio-metabolic health were assessed at baseline and post-intervention. Measures of dietary compliance, mood, hunger and satiety were assessed daily throughout the 12 week intervention.

4.2 Participants

Participants were recruited through advertising on social media channels targeting current and past university students in Victoria, Australia. A total of 194 individuals responded to the advertisement and underwent initial screening. Only 44 were deemed eligible and were recruited for the study. Participants were eligible for inclusion if they: (i) were aged between 18-45 years; (ii) had a body mass index (BMI) of 22.0-35.0kg/m^2; (iii) had a body fat percentage >18% for males or >25% for females as measured via dual x-ray absorptiometry (DXA); (iv) had not followed a structured resistance training program in the previous 6 months and; (v) had been weight stable for 3 months prior to the study (<5% weight loss or weight gain). Participants were excluded if they: (i) were smokers; (ii) had diabetes; (iii) had a history of cardiovascular disease; (iv) were taking dietary supplements and were unwilling to cease these for the duration of the study; (v) were taking glucose or lipid lowering, or weight loss medication; (vi) had a current physical condition that may have been exacerbated by resistance training as determined by their general practitioner, (vii) were pregnant or intended to become pregnant in the following 3-4 months; (viii) were menopausal or post-menopausal; (ix) had a history of disordered eating; (x) had a current or previous respiratory condition likely to be exacerbated by the intervention; (xi) had a current or previous gastrointestinal disorder likely to be exacerbated by the intervention; (xii) had any allergy to any components of the supplement product to be supplied; (xiii) were unable to commit to fasting on assigned days if randomised to the IFT group; (xiv) did not speak English at a level at which they were able to understand and complete the requirements of the study or; (xv) disclosed any other chronic disease or condition, or were taking any other medication that investigators deemed would contraindicate the study intervention.

4.3 Randomisation

Participants who were eligible for the study were stratified by age, sex and BMI before being randomised by coin toss into either the IF plus resistance training (IFT) or CER plus resistance training (CERT) group for 12 weeks. These parameters were chosen for matching as they are practical to use at an early stage of the screening process, and it is believed these are likely to have the greatest impact on body composition changes as baseline variables. The intervention took place from February to November, 2019, spread across 6 groups, starting 2-4 weeks apart. This study was approved by the Swinburne University of Technology Human Research Ethics Committee (project #2018/322). A copy of ethics approval for this project can be seen in Appendix 5.

4.4 Dietary intervention

Basal energy requirements for all participants were calculated using the Mifflin St. Jeor equation (109), with total energy requirements calculated by applying an activity factor of 1.4 representing a recreational level of activity (based on prescribed exercise). At the beginning of the intervention period, all participants were provided with example meal plans that would result in consumption of approximately 80% of estimated energy requirements and 1.4 grams of protein per kilogram of body weight per day (g/kg/day). Meal plans were customised based on food preferences for each individual and provided by the PhD candidate, who is a trained dietitian, along with brief education on the Australian healthy eating guidelines. An example meal plan can be found in Appendix 6. As participants in the IFT group had limited energy available on fasting days to reach recommended protein intakes, they were provided with high protein shakes and high protein soups in order to get as close to recommendations as possible. Participants were also instructed on how to use the Easy Diet Diary (Xyris Software, Australia, 2019) smart phone application to record their food, and substitute other foods of their choosing into the meal plans, while maintaining the same energy and protein intake.

4.4.1 Intermittent fasting diet protocol

All participants in the IFT group were instructed to consume 100% of their energy requirements for 5 days per week (non-fasting days). On two non-consecutive and non-training days, participants consumed approximately 30% of their estimated energy requirements (~2,100 kJ for females and ~2,500 kJ for males) (fasting days), consistent with previous research (73,98). On fasting days, participants were prescribed a diet consisting of whey-based high protein meal replacement shakes (Formulite), high-protein soups and steamed/raw vegetables. The macronutrient composition of these supplements and the recommended intake on fasting days can be seen in Table 4.1. On fasting days, participants were asked to consume all energy during a 6 hour window between 12.00 pm and 6.00 pm and were allowed ad libitum consumption of non-energy providing beverages. In order to match protein intakes across dietary groups as closely as possible, those in the IFT group were instructed to consume approximately 1.5 g/kg/day of protein on non-fasting days, as their fasting day diets only provided ~1.1-1.2 g/kg/day.

Table 4.1. Composition of fasting day meals consumed by IFT group and overall intake on fasting days.

	Male	**Female**
Foods	2 x meal replacement shakes 1 x high protein soup 150g raw/steamed vegetables	1.5 x meal replacement shakes 1 x high protein soup 150g raw/steamed vegetables
Nutrients		
Energy (kJ/kcal)	2511/597	2080/495
Protein (g)	93.6	76.7
Carbohydrates (g)	30.4	26.3
Fat (g)	10.0	8.0

4.4.2 Continuous energy restriction dietary protocol

Those randomised to the CERT group were instructed to consume ~80% of their total energy requirements daily for the duration of the 12 week intervention. Furthermore, participants were also required to consume 1.4 g/kg/day of protein. Participants in this group also received customised meal plans and the same education as those in the IFT group.

4.5 Exercise intervention

All participants were required to undertake 3 training sessions each week: 2 resistance training sessions and 1 body weight aerobic/resistance training combination session. The two resistance training sessions were conducted at Swinburne University's Hawthorn campus, and were supervised by the PhD candidate who is an accredited strength and conditioning coach (and was also the study dietitian). The 2 supervised sessions consisted of variations of the following exercises: push-ups, squats, rows, lunges, bicep curls and dips. Participants completed these exercises in a superset style workout. Participants aimed to complete 12-15 repetitions of one exercise in the superset, followed immediately by the other exercise, before being allowed a 2 minute break. This was repeated 3 times before moving on to the next superset. Superset 1 consisted of push ups and squats; superset 2 consisted of rows and lunges; superset 3 consisted of bicep curls and dips. Once participants were able to complete 3 sets of 15 repetitions in any individual exercise, the weight was increased or exercise variation made more difficult, adhering to the principles of progressive overload. The one body weight aerobic/resistance training combination session per week was completed by participants at home using body weight exercises consisting of: planks, mountain climbers, crunches, burpees, lying side toe-touches and hip bridges. These exercises were also completed using a superset format with 2 minute breaks, however were timed instead of counting repetitions. When participants reached their time goal with good form (self-assessed), they were instructed to increase this by 5 seconds. All exercises utilised are shown in Appendix 7.

4.6 Baseline, during and end of intervention testing

4.6.1 Body composition analysis

Body composition was analysed at baseline and end of intervention using a number of methods. DXA was utilised to assess overall changes in LBM, body fat and body fat percentage. Ultrasound assessment of the mid-thigh region was undertaken to assess changes in thickness, circumference and cross sectional area of the rectus femoris, and the thickness of the vastus intermedius. Furthermore, ultrasound images were used to determine echogenicity of these areas as an assessment of change in muscle quality. Peripheral quantitative computed tomography (pQCT) was conducted on the mid-thigh region, to analyse quantitative changes in muscle tissue and fat tissue in this area. Bioelectrical impedance analysis was conducted on a weekly basis to assess changes in body mass, body fat and LBM across the 12 week study period in order to provide ongoing feedback to the participants.

4.6.2 Dual x-ray absorptiometry

LBM, fat mass and body fat percentage were assessed utilising DXA [Hologic Horizon (Bedford MA)] at baseline and after the intervention period, utilising a previously detailed protocol (110). The DXA was calibrated for bone mineral density, muscle and fat masses on the morning of each assessment in accordance with manufacturer guidelines using spine and whole-body phantoms respectively. DXA was chosen as the primary method of assessing body composition changes for two reasons. First, it is a tool that has been commonly utilised for assessing changes in body composition during periods of weight loss in a diverse range of populations (111–114). Second, DXA is a widely available, safe and cost-effective method of assessing changes in body composition (115,116). However, it is noted that there are a number of limitations to the use of DXA for assessing changes in body composition. Firstly, the accuracy of DXA may be impacted by initial weight and body thickness, especially in obese individuals (117). Secondly, the ability of DXA to accurately assess changes in body composition longitudinally has been questioned when compared to more accurate models (i.e. the 4 compartment model) (118). Nonetheless, DXA is often cited as the gold-standard for assessing changes in body composition, and despite error associated with intra-individual assessment (118), shows strong correlations with more accurate methods for assessing changes in body composition at the group level (119). In order to obtain reliable results, standardised presentation of subjects were used at each scan. To achieve this, the following procedure was followed for each body composition scan:

1. All scans were completed in the morning, prior to 12pm;
2. Participants were asked to collect a urine sample upon arrival for hydration testing;
3. Participants were asked to present to the Western Centre for Research and Education in a fasted state (fasted for a minimum of 8 hours) - no food or fluid ingestion (except for small sips of water if needed) after 11pm on the night before the scan;
4. Participants were asked to ensure they did not undertake any exercise on the morning of the scan;
5. Participants were be asked to void their bladder immediately prior to the scan;
6. Participants were asked to wear light clothing free from metal (plain t-shirt and shorts, with no wired undergarments), and asked to remove all jewellery;
7. Participants were asked to lay down in a supine position in the centre of the scanning bed. The researcher/technician aligned them in the centre of their bed with a straight

spine. Their feet were internally rotated and held in place with tape. Their hands were flat against the scanning bed with fingers together (see Figure 4.1 for positioning);

8. The participant was asked to refrain from moving or talking for the duration of the scan (approximately 7 minutes);

9. Once completed, the researcher/technician adjusted the regional analysis by the software as necessary;

Figure 4.1. Positioning of participants on the DXA scanning bed*

*Note participant's feet have not yet been taped.

4.6.3 Ultrasound

Ultrasonography was utilised in order to assess changes in local muscular hypertrophy and quality of the mid-thigh region in addition to DXA-derived whole body composition changes. While DXA provides an estimate of changes in total LBM and fat mass, it is unable to identify or quantify local changes in cross-sectional area (CSA) and thickness of muscles, or changes in muscle quality, measures that may have important implications for physical strength, function and healthy ageing (120,121). Thus, muscle thickness, cross-sectional area (CSA) and muscle quality [echogenicity (EI)], were measured using ultrasound (SonoSite M-Turbo, SonoSite Australasia Pty Ltd, New South Wales, Australia) with a linear array transducer (5-2 MHz) for the rectus femoris (RF) and vastus intermedius (VI) of the non-dominant leg at baseline and post intervention in the majority of the participants. These muscles were chosen in order to capture changes in muscles that contain greater proportions of fast twitch fibres involved in powerful movements (RF) and those that contain greater proportions of predominantly slow-twitch fibres involved in stability and endurance (VI), which are known to adapt differently to resistance training (122). All images were acquired and analysed by the same technician. Varying depths were required to obtain full visualisation of the RF in some instances, however gain was kept consistent across measurements. Measurements were acquired with participants in a supine position, with their knee in passive extension. Ultrasound gel was applied to the transducer, which was placed perpendicular to the long axis of the anterior thigh, at a distance of two thirds from the anterior, superior iliac spine to the superior patellar border, consistent with previous studies (121) (Figure 4.2). Muscle thickness and CSA were measured in real-time with the on-board functions of the M-turbo, utilising the straight line and tracing functions respectively (Figure 4.3). Images were also saved onto the on-board hard-drive before being transferred onto a personal computer for EI analysis using ImageJ software (NIH, Bethesda, MD) (123). EI for RF and VI were measured utilising a standard square of 100 x 100 pixels, or where the predefined square did not fit within the cross section of the muscle, the largest square that fit within the anatomic boundaries of the muscle was utilised, a method which has shown good inter-observer reliability regardless of level of expertise (124) (Figure 4.4).

Figure 4.2. Positioning of the ultrasound probe.

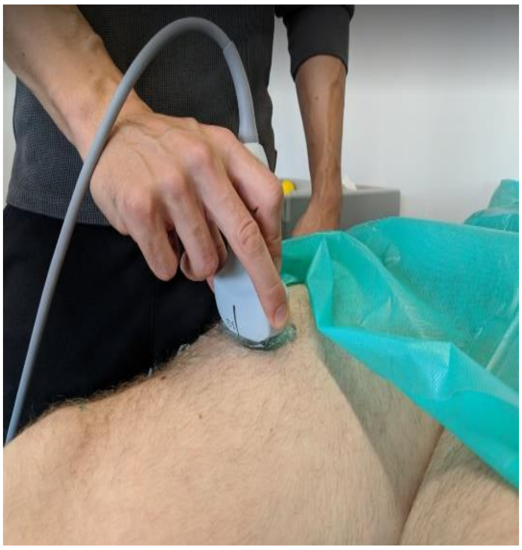
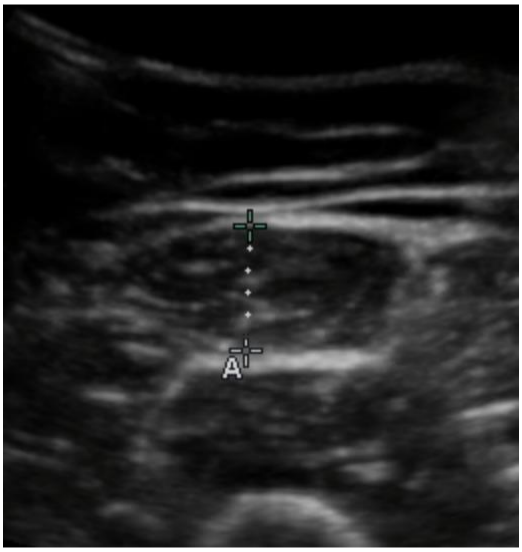
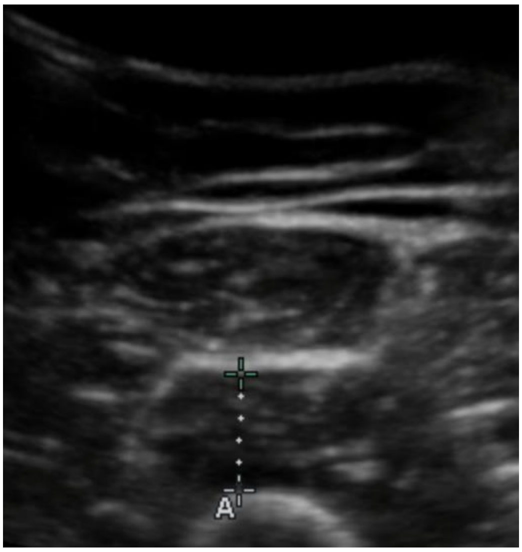


Figure 4.3. Example of ultrasound analysis of: a) rectus femoris thickness; b) vastus intermedius thickness; c) rectus femoris cross-sectional area and; d) rectus femoris echogenicity.

a)



b)



b)

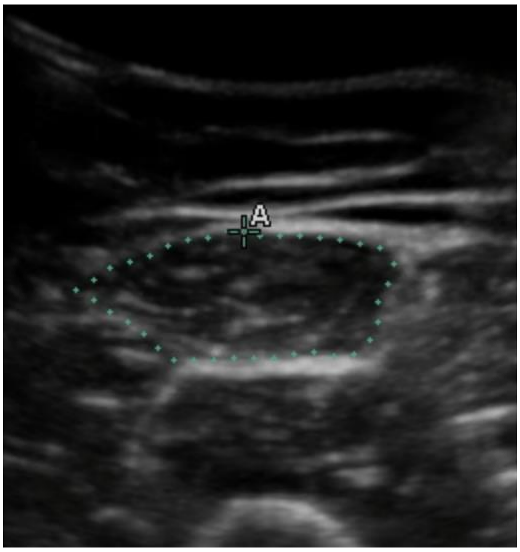
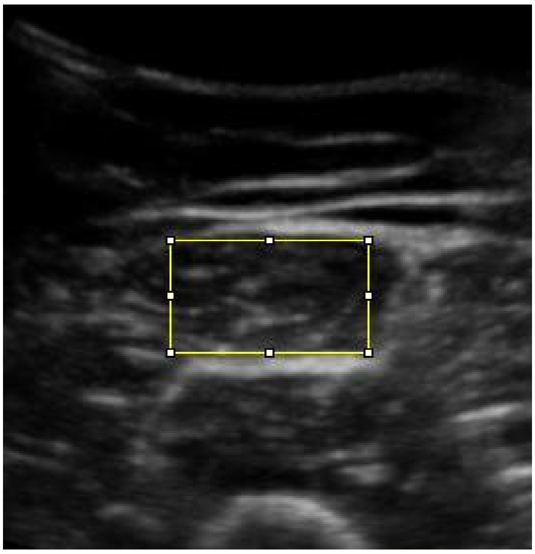
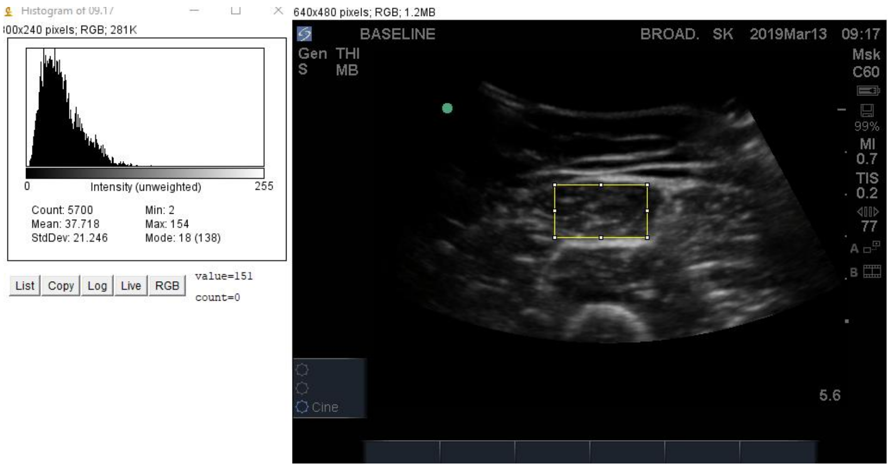
c)  d) 

Figure 4.4. Assessment of echogenicity using ImageJ software.



4.6.4 Peripheral quantitative computed tomography (pQCT)

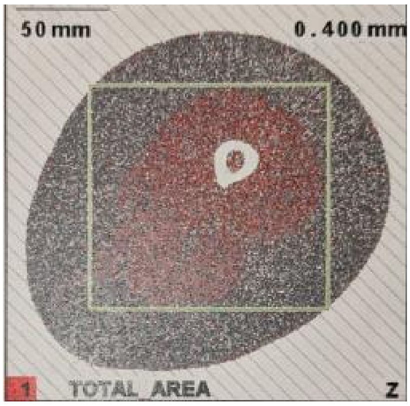
pQCT utilises x-rays in order to provide a high-resolution cross-sectional image of the region of interest. Cross-sectional muscle area measured by pQCT has been shown to correlate highly with measurements derived from magnetic resonance imaging (125), considered to be the gold standard for imaging skeletal muscle (116). Additionally, pQCT images allow quantification of both subcutaneous and intramuscular adipose tissue, although precision for the latter has been shown to vary considerably (126). Nonetheless, increased intramuscular fat measured via pQCT (indicating lower muscle density) has been associated with greater mortality in older individuals (127) and risk of type 2 diabetes in younger cohorts (128). Given the additional information provided by pQCT, this was employed as another measure of assessing changes in skeletal muscle.

At baseline and post-intervention, pQCT was utilised to measure the surface area of muscle, intramuscular fat and subcutaneous fat of the mid-thigh region. As this site is not often utilised for pQCT scans (normally the forearm or foreleg are scanned), the set-up of the pQCT machine needed to be customised (Figure 4.5). This involved sourcing a chair wide enough to fit over the gantry on which the pQCT sat.

Figure 4.5. pQCT set up for scanning the mid-thigh region.

A single 2.5mm transverse pQCT; (Stratec XCT3000, Stratec Medizintechnik GmbH, Pforzheim, Germany) scan with a voxel size of 0.4mm was obtained at mid-thigh region of the non-dominant leg. The mid-thigh was defined as midway between the tip of the greater trochanter and medial edge of the tibial plateau, located by deep palpation. The images were exported and further analyzed by Slice-O-Matic^{TM} (Tomovision, Montreal, CA) to determine the muscle, intramuscular fat and subcutaneous fat volumes as previously described (129–131). An example of the scan output prior to analysis can be seen in Figure 4.6. After visual checks; where due to beam hardening artefacts the tissue was not segmented ("tagged") optimally, the assignment of individual voxels or small voxel islands were changed into the correct tissue manually, at the discretion of the operator. All imaging and image analyses were carried out by a single experienced image analysis specialist or under his direct supervision and quality control.

Figure 4.6. Output from transverse pQCT scan of the mid-thigh prior to further analysis.



4.6.5 Bioelectrical impedance analysis

Utilising a set of Tanita MC780-U scales, participants had their weight, body water, body fat and LBM measured on a weekly basis using multi-frequency segmental body composition analysis. Prior to their first exercise session of each week, participants were asked to stand barefoot and in light clothing on the metal contacts of the metal foot pads and hold the hand-grips with arms relaxed by their sides. Their height, sex and age were entered into the scales, and after analysis, body fat percentage, body fat mass, LBM percentage, LBM, water mass, water mass percentage and BMI were recorded. This data was collected in order to provide to participants weekly feedback and encourage greater compliance to the dietary protocol.

4.6.6 Strength testing

Strength testing was undertaken prior to the diet intervention and at the end of week 12. A 3 repetition maximum (3RM) test and strength endurance test were performed for both bench press and leg press. After a brief 5 minute warm up, participants were instructed on correct lifting and breathing techniques before practicing these using submaximal loads for 10-15 repetitions (Figure 4.7). Weight was gradually added and repetitions reduced to serve as a functional warm up. Participants then completed a set of 3 repetitions at a self-selected weight close to their perceived capacity, followed by a 3 minute rest, with weight being continually added to each subsequent attempt. 3RM was recorded as the last successful attempt before form breakdown, or failure to complete the lift without assistance. The 3RM of each participant was determined within 5 attempts. After the 3RM test, participants were allowed a 5 minute rest before undergoing a strength endurance test. These were tested in order such that 3RM bench press was followed by the bench press endurance test, and 3RM leg press was followed by the leg press endurance test. Participants were required to complete as many repetitions as possible of each exercise utilising 70% of their estimated 1 repetition maximum, calculated from the attained 3RM utilising the Brzycki formula (132) (weight lifted/[1.0278 – (0.0278 x repetitions performed)]). Failure was determined as the first repetition where the participant required assistance. Repetitions where form was considered inadequate were not counted; however, participants were not stopped from completing subsequent repetitions if this occurred. Volume was calculated as the number of repetitions completed at 70% of 1 repetition maximum multiplied by weight lifted.

Figure 4.7. Warm up for the bench press 3 repetition maximum test

4.6.7 Blood testing

Fasted blood serum samples were taken from each participant prior to and after the completion of the 12 week intervention in order to assess the impacts of the intervention on blood markers of cardio-metabolic health. Samples were taken between 7.00 am and 11.00 am after a minimum 8 hour fast (although participants were encouraged to consume water on the night before and the morning of testing). Approximately 20 mL of blood was drawn into a serum separating tube from a vein in the antecubital region. The tube was left to sit for at least 20 minutes, but less than 40 minutes, to allow clotting. The samples were then centrifuged for 12 minutes at 3000 RPM in order to separate the serum. Serum was then aliquoted and stored at -84 degrees Celsius before being packed in dry ice and sent via courier to an external lab for analysis (Melbourne Pathology, Victoria, Australia). Serum samples were analysed for total serum cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides, blood glucose, insulin and high-sensitivity C-reactive protein (hsCRP). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the equation ([blood glucose x insulin]/22.5) (133).

4.6.8 Dietary compliance, mood, hunger and satiety and post-intervention intentions

For each day of the 12 week intervention, participants were asked to complete an online survey. Each participant received a unique link to a survey that included 17 questions in total, pertaining to level of hunger, satiety, energy levels, mood and compliance; these questions can be found in Appendix 8. These questions were adapted from previously validated visual analogue scales (134,135) into 0 to 10 point Likert scales in order to facilitate use on a mobile phone. Four questions pertained to hunger, 4 to cravings, 4 to energy levels and 4 to mood. Additionally, there was a final question asking participants to indicate on a scale of 0 to 10 how compliant they felt they were with their diet that day, based on meeting their pre-defined energy and protein requirements. For analysis, these questions were combined into 5 distinct measures; hunger, cravings, energy levels, mood and compliance which can be seen in Table 4.2.

Table 4.2. Questions included within each survey category for hunger, cravings, mood and energy levels.

Hunger (Maximum score possible 40)	**Cravings (Maximum score possible 40)**
1. How hungry do you feel? (+ve) 2. How satisfied do you feel? (-ve) 3. How full do you feel? (-ve) 4. How much do you think you can eat? (+ve)	1. Would you like something sweet? (-ve) 2. Would you like something salty? (-ve)

	3. Would you like something savoury? (-ve) 4. Would you like something fatty? (-ve)
Energy levels (Maximum score possible 40) 1. How alert do you feel? (+ve) 2. How much of an effort is it to do anything? (-ve) 3. How weary do you feel? (-ve) 4. How sleepy do you feel? (-ve)	**Mood (Maximum score possible 40)** 1. How sad do you feel? (-ve) 2. How tense do you feel? (-ve) 3. How happy do you feel? (+ve) 4. How calm do you feel? (+ve)

Scoring for each measure was done by combining the responses (0-10) from the Likert scale for each of the 4 questions. In order to represent these overall scores as positive values for easier interpretation, for those questions where a higher score indicated a negative response (eg. How sleepy do you feel?), were transformed by subtracting the recorded Likert scale score from 10, thus each question had a maximum score of 40, except for compliance which had a maximum score of 10. Furthermore, as the original cravings questions were validated in such a way that higher scores indicated lower cravings, all questions for this section were transformed (as above) when presenting results to ensure easier interpretation. Thus, higher scores on the cravings scale presented in the data represent higher levels of cravings. Participants were asked to complete the survey just prior to going to bed each day, consistent with previous research (136). In order to promote completion of the survey, participants were sent up to 5 text message reminders per week.

Finally, at the end of the intervention, participants were asked to rate how easy they found the diet to comply with on a scale of 0 to 10, whether they thought they would continue with the diet after the intervention had finished, and whether or not they would have preferred to be in the alternate intervention group (Appendix 9). Participants were also asked to explain what they felt the most difficult part of the intervention was.

4.6.9 Dietary intake

Participants were required to keep a 3 day food diary at baseline and in week 1, 6 and 12 using the Easy Diet Diary (Xyris Software, Australia, 2019) phone application. Participants recorded all food and drink intake on non-consecutive days that included 2 weekdays and 1 weekend day. Analysis was conducted using the Easy Diet Diary Connect website, with values checked for accuracy (137). Food records were kept on non-fasting days for those in the IFT group. On fasting days, participants were asked to note down any extra food or drink consumed, and whether they had consumed their recommended supplements. Intake on these days was estimated from these records.

Conclusion to chapter

This chapter provided a detailed description of all methods utilised in the research study and forms the basis of the methods used in the following chapters. It is hoped that by including this as a stand-alone chapter, the reader will be able to better conceptualise the project when reading through subsequent chapters.

Chapter Five

Chapter Five: Effect of intervention on body composition, muscle size, quality and strength.

5.1 Preface to chapter

The following chapter is written as an extended manuscript and aims to answer the first research question: are there differential effects on LBM, muscle quality and size or strength when undertaking a resistance training program in conjunction with 5:2 fasting compared to CER?

A shorter version of this chapter (due to journal word limits) has been submitted for publication, please see Appendix 10:

Publication Details: **Keenan, S**, Cooke, MB, Bani Hassan, E, Chen, WS, Sullivan, J, Wu, SX, El-Ansari, D, Imani, M, Belski, R, (2020). Intermittent fasting and continuous energy restriction result in similar changes in body composition and muscle strength when combined with a 12 week resistance training program. *Journal of Nutrition*. **Under review**.

5.2 The effects of the intervention on body composition, muscle size and strength

Intermittent fasting and continuous energy restriction result in similar changes in body composition and muscle strength when combined with a 12 week resistance training program

Abstract

Background: Energy restricted diets commonly lead to loss of lean body mass (LBM), however resistance training and increased protein intake can help attenuate these losses.

Objective: The objective of this study was to compare the effects of 12 weeks of resistance training combined with either 5:2 intermittent fasting or continuous energy restriction on body composition, muscle size and quality, and upper and lower body strength.

Methods: Untrained individuals were randomly assigned to resistance training plus either continuous energy restriction [20% daily energy restriction (CERT)] or 5:2 intermittent fasting [~70% energy restriction 2 days/week, euenergetic consumption 5 days/week (IFT)] groups, with both groups prescribed an average of ≥ 1.4 grams of protein per kilogram of body weight per day. Participants completed 2 supervised resistance and 1 unsupervised aerobic/resistance training combination sessions per week for 12 weeks. Changes in LBM were assessed as a primary outcome. Other body composition changes, thigh muscle size and quality, strength and dietary intake were assessed as secondary outcomes.

Results: Thirty-four participants completed the study (CERT; n = 17, IFT; n = 17). At the end of the 12 weeks, LBM was significantly increased (+3.7%, $p<0.0001$) and both body weight (-4.6%, $p = <0.0001$) and fat (-24.1%, $p<0.0001$) were significantly reduced with no difference between groups, though results differed by sex. Both groups showed improvements in thigh muscle size and quality, and reduced intramuscular and subcutaneous fat assessed by ultrasonography and peripheral quantitative computed tomography (pQCT), respectively. However, only the CERT group demonstrated a significant increase in muscle surface area assessed by pQCT. Similar gains in upper and lower body strength and muscular endurance were observed in both groups.

Conclusion: When combined with resistance training and moderate protein intake, continuous energy restriction and 5:2 intermittent fasting resulted in similar improvements in body composition, muscle quality, and strength.

Key words: Intermittent fasting; continuous energy restriction; resistance training; body composition; lean body mass; intramuscular fat; weight loss.

Introduction

Energy restricted diets are increasingly popular amongst individuals for a variety of reasons ranging from improving body composition to general health and wellbeing. Regardless of the reason, these diets commonly lead to weight loss in the form of both fat and lean body mass (LBM) (17). While fat loss is usually desirable, reductions in skeletal muscle mass (a major component of LBM) may lead to a number of deleterious short- and long-term consequences, such as hyperphagia and reduced BMR, which may compromise long term weight loss success (11); increased risk of strength loss and disability, especially in older adults (5,138); and potential metabolic issues incumbent with low muscle mass (139).

The mechanisms behind weight-loss induced reductions in LBM are not fully understood, however, the impact of energy restriction on protein turnover and net muscle protein balance may be a contributing factor (140). Skeletal muscle mass is determined by the balance of muscle protein synthesis (MPS) and muscle protein breakdown (MPB), which remains equal during energy balance (141). Conversely, during short-term, continuous energy restriction, both post-prandial and post-absorptive MPS are reduced (142), which may lead to an overall negative protein balance, higher protein catabolism to supply amino acids and reductions in muscle mass (140). However, whether this also occurs over extended periods of energy restriction is unclear (143). Notwithstanding, higher protein intakes, and/or performing resistance training have been shown to partially or completely attenuate these reductions in MPS (140,144). Moreover, these strategies, as well as others including slower rates of weight loss, have been utilised to successfully mitigate LBM loss during longer periods of energy restriction (17,145).

Recently, the pattern of energy restriction has emerged as another potential method for preserving LBM. Compared to traditional energy restricted diets that are characterised by moderate daily energy restriction (i.e. continuous energy restriction), alternative patterns such as intermittent fasting, which intersperse periods of severe energy restriction with regular dietary or ad libitum consumption have gained recent attention. It has been hypothesised that intermittent fasting may be protective of LBM loss compared to continuous energy restriction (146), though some argue that severe energy restriction, even if acute, could lead to greater reductions in LBM (147). Notwithstanding, it is clear that the majority of energy restricted diets, regardless of the pattern, can still lead to LBM loss (148), and thus should be combined with other strategies that are known to preserve and/or promote muscle growth.

While there are many variations of intermittent fasting, the 5:2 style diet, which generally involves 2 days per week of severe (consumption of ~1,600-3,000 kJ/day) or complete energy restriction, paired with 5 days of ad libitum or euenergetic consumption (29,91–95,103–105,107,108,149), has received limited attention in the context of LBM preservation. To date, no studies have compared the effects of a 5:2 style diet to continuous energy restriction on body composition and muscle strength adaptations when both are combined with a resistance training program. Furthermore, only a handful of intermittent fasting studies have utilised more sensitive assessments of muscle hypertrophy (i.e. ultrasonography) to simultaneously assess changes in muscle growth (25,101,102,150). Thus, the purpose of this study was to investigate and compare the effects of 12 weeks of resistance training combined with either a 5:2 fasting or continuous energy restriction style diet on body composition, especially LBM, indicators of muscle hypertrophy and quality, and upper and lower body strength.

Methods

Detailed study methods are presented in Chapter 4. The methods for the statistical analysis relating to the results presented in this chapter are presented below.

Statistical Analysis

Results are presented as mean (± SD). Normality was assessed using the Shapiro-Wilk test and visual inspection of Q-Q plots. Assumptions of normality were violated for intramuscular fat only. Intramuscular fat was log10 transformed, resulting in normality. Linear mixed models were used to analyse variables for main effects of time and group, and time x group, time x sex and time x group x sex interactions. Differences between groups at baseline were analysed using independent t-tests. Bivariate correlations using Pearson's correlation co-efficient were calculated to assess relationships between variables and changes in LBM. All analyses were performed using SPSS version 25 (IBM Corp, Armonk, NY, 2017). A *p*-value of <0.05 was considered significant for all tests.

Results

Participant characteristics

There were a total of 17 completers in each group, with a nearly even split of males and females (IFT = 9 males and 8 females, CERT = 8 males and 9 females). Overall, 10 participants (n = 8 female and n = 2 male) failed to complete the interventions (CERT = 5, IFT = 5). Of these 10, 3 were unable to commit to the exercise sessions, 2 were unable to commit to the dietary protocol (IFT = 1, CERT = 1), and the remaining 5 dropped out due to unrelated medical issues or relocation. A flow-chart showing participant movement through the study is presented in Figure 1. Baseline characteristics for participants are presented in Table 5.1. No significant differences were found between groups as a whole, or when split by sex (not reported).

Table 5.1. Baseline participant characteristics

Baseline variables	IFT Males (n = 9)	CERT Males (n = 8)	IFT Females (n = 8)	CERT Females (n = 9)	*p*-value[1]
Age (years)	25.2 (6.2)	23 1 (2.6)	24.3 (2.9)	23.2 (4.9)	0.31
Height (cm)	181.1 (0 1)	178.6 (0.1)	161.6 (0.1)	164.2 (0.1)	0.82
Weight (kg)	87.3 (12 5)	88.0 (11.5)	71.9 (10.7)	72.1 (10.6)	0.92
BMI (kg/m^2)	26.6 (3.0)	27.6 (2.4)	27.5 (2.5)	26.7 (3.4)	0.93
LBM (kg)	64.4 (7.2)	64.6 (9.4)	43.0 (5.6)	43.2 (4.8)	0.82
Body Fat Percentage (%)	29.4 (6.0)	30.4 (3.7)	43.0 (3.9)	42.2 (6.3)	0.76
Bench press 3RM (kg)	58.0 (12 1)	57.0 (12.9)	26.8 (4.9)	23.9 (5.6)	0.56
Bench press volume (70% 1RM) (kg)	610.6 (197.0)	522.1 (127.6)	283.4 (104.1)	247.2 (66.3)	0.26
Leg Press 3RM (kg)	150.6 (37.1)	165.0 (47.9)	68.8 (32.8)	57.4 (22.8)	0.85
Leg press volume (70% 1RM) (kg)	1636.2 (781.6)	1468.3 (578.0)	759.4 (558.1)	582.0 (298.4)	0.37

Note: Mean (*SD*). [1]P-values reported are for independent t-tests between intervention groups. BMI = body mass index, LBM = lean body mass, 1RM = 1 repetition maximum, 3RM = 3 repetition maximum.

Body weight and body composition analysis

Body weight and body composition measured before and after the intervention are presented in Table 5.2. There was a main effect for time for weight [F(1, 30) = 72.25, *p*<0.001], BMI [F(1, 30) = 65.45, p<0.001], body fat mass [F(1, 30) = 150.00, *p*<0.001], body fat percentage [F(1, 30) = 215.69, *p*<0.001] and LBM [F(1, 30) = 42.47, *p*<0.001], with significant reductions in weight, BMI, body fat mass and body fat percentage observed in both dietary groups, whereas LBM was significantly increased in both groups. A significant time x sex interaction was evident for weight [F(1, 30) = 13.12, *p*=0.001], BMI [F(1, 30) = 8.47, *p*=0.007] and LBM [F(1, 30) = 10.41, *p*=0.003], with larger reductions in weight and BMI observed in males, but greater increases in LBM observed in females.

Mid-thigh muscle surface area and intramuscular and subcutaneous fat analysis

pQCT

Muscle surface area and intramuscular and subcutaneous fat measured before and after the intervention via pQCT are reported in Table 5.2. A main effect for time was found for subcutaneous fat [F(1, 27) = 21.26, *p*<0.001] and log10 intramuscular fat [F(1, 27) = 7.13, *p*=0.01] with significant reductions occurring in both groups over time. There was a time x group effect for muscle surface area, with those in the CERT group experiencing a mean increase in muscle surface area compared to the IFT group [F(1, 27) = 5.65, *p*=0.03].

Ultrasound

RF thickness, CSA and EI, and VI thickness and EI measured before and after the intervention via ultrasound are presented in Table 5.2. A main effect for time for RF thickness [F(1, 23) = 36.90, *p*<0.001], RF CSA [F(1, 23) = 44.35, *p*<0.001] and RF EI [F(1, 23) = 17.18, *p*<0.001] was noted, with RF thickness and CSA significantly increased in both groups over time, whereas RF EI significantly decreased in both groups over time. There were no other significant interactions or main effects identified for RF measurements and/or all VI assessments.

Table 5.2. The effects of 12 weeks of IFT and CERT on body weight, BMI, body composition, pQCT and ultrasound variables in male and female participants.

	Group	Baseline	Week 12	Δ	Δ (%)	*P* (group)	*P* (time)	*P* (I)	*P* (S)
Body composition variables									
BMI (kg/m^2)	IFT Males (n=9)	26.6 (3.0)	25.1 (2.7)	-1.5	-5.6	0.98	<0.001[1]	0.64	0.007[3]
	CERT Males (n=8)	27.6 (2.4)	25.5 (2.7)	-2.1	-7.6				
	IFT Females (n=8)	27.5 (2.5)	26.5 (2.5)	-1.0	-3.6				
	CERT Females (n=9)	26.7 (3.36)	26.0 (2.9)	-0.7	-2.6				
Weight (kg)	IFT Males (n=9)	87.3 (12.5)	82.6 (11.7)	-4.7	-5.4	0.97	<0.001[1]	0 55	0.001[3]
	CERT Males (n=8)	88.0 (11.5)	81.7 (12.1)	-6.3	-7.2				
	IFT Females (n=8)	71.9 (10.7)	69.5 (9.8)	-2.4	-3.3				
	CERT Females (n=9)	72.1 (10.6)	70.1 (8.8)	-2.0	-2.8				
LBM (kg)	IFT Males (n=9)	64.4 (7.2)	65.7 (7.4)	1.3	2.0	0.99	<0.001[1]	0.47	0.003[3]
	CERT Males (n=8)	64.6 (9.4)	65.0 (9.6)	0.4	0.6				
	IFT Females (n=8)	43.0 (5.6)	45.4 (5.6)	2.4	5.6				
	CERT Females (n=9)	43.2 (4.8)	45.8 (4.1)	2.6	6.0				
Fat mass (kg)	IFT Males (n=9)	27.4 (8.9)	20.2 (6.3)	-7.2	-26.3	0.96	<0.001[1]	0 32	0.18
	CERT Males (n=8)	28.2 (5.0)	19.3 (4.8)	-8.9	-31.6				
	IFT Females (n=8)	32.8 (6.9)	26.0 (5.1)	-6.8	-20.7				
	CERT Females (n=9)	32.3 (8.8)	26.2 (7.9)	-6.1	-18.9				
Body fat percentage (%)	IFT Males (n=9)	29.4 (6.0)	23.1 (4.6)	-6.3	-21.4	0.95	<0.001[1]	0.64	0.75
	CERT Males (n=8)	30.4 (3.7)	22.8 (4.3)	-7.6	-25.0				
	IFT Females (n=8)	43.0 (3.9)	36.2 (2.2)	-6.8	-15.8				
	CERT Females (n=9)	42.2 (35.9)	35.9 (6.7)	-6.3	-14.9				
pQCT variables									
Muscle (cm^2)	IFT Males (n=7)	172.5 (23.2)	170.8 (23.4)	-1.7	-1.0	0.40	0.10	0.03[2]	0.21
	CERT Males (n=8)	162.5 (17.7)	165.2 (18.2)	2.7	1.7				
	IFT Females (n=8)	117.9 (21.3)	118.1 (17.8)	0.2	0.2				
	CERT Females (n=8)	111.3 (12.2)	118.1 (8.1)	6.8	6.1				
Intra-muscular fat (cm^2)[4]	IFT Males (n=7)	1.03 (0.64)	0.68 (0.58)	-0.35	-33.98	0.61	0.01[1]	0.63	0.70
	CERT Males (n=8)	2.30 (2.11)	1.33 (1.21)	-0.97	-42.17				
	IFT Females (n=8)	2.22 (1.08)	1.48 (0.78)	-0.74	-33.33				
	CERT Females (n=8)	1.71 (1.00)	1.40 (0.71)	-0.31	-18.13				
Subcutaneous fat (cm^2)	IFT Males (n=7)	71.2 (10.5)	63.7 (18.3)	-7.5	-10.5	0.41	<0.001[1]	0.62	0.83
	CERT Males (n=8)	79.7 (27.5)	64.9 (16.3)	-14.8	-18.6				
	IFT Females (n=8)	134.8 (35.8)	116.3 (33.8)	-18 5	-13.7				

	CERT Females (n=8)	142.6 (42.9)	136.6 (48.3)	-6.0	-4.2				
Ultrasound variables									
RF Thickness (cm)	IFT Males (n=7)	1.83 (0.36)	1.98 (0.31)	0.15	8.20	0.73	<0.001[1]	0.05	0.09
	CERT Males (n=5)	1.95 (0.27)	2.17 (0.28)	0.22	11.28				
	IFT Females (n=6)	1.78 (0.15)	1.82 (0.24)	0.04	2.25				
	CERT Females (n=9)	1.49 (0.26)	1.64 (0.27)	0.15	10.07				
RF CSA (cm^2)	IFT Males (n=7)	6.37 (1.09)	7.43 (1.37)	1.06	16.64	0.35	<0.001[1]	0.07	0.11
	CERT Males (n=5)	6.85 (1.50)	7.96 (1.32)	1.11	16.20				
	IFT Females (n=6)	6.16 (1.19)	6.19 (1.29)	0.03	0.49				
	CERT Females (n=9)	4.28 (1.23)	5.14 (1.46)	0.86	20.09				
RF EI (arbitrary units)	IFT Males (n=7)	22.1 (8.2)	15.9 (4.5)	-6.2	-28.1	0.43	<0.001[1]	0.47	0.95
	CERT Males (n=5)	17.1 (6.6)	14.5 (3.7)	-2.6	-15.2				
	IFT Females (n=6)	33.4 (14.0)	29.1 (15.0)	-4.3	-12.9				
	CERT Females (n=9)	42.0 (10.6)	38.3 (11.3)	-3.7	-8.8				
VI Thickness (cm)	IFT Males (n=7)	1.94 (0.37)	1.78 (0.20)	-0.16	-8.25	0.50	0.38	0 21	0.12
	CERT Males (n=5)	1.63 (0.22)	1.55 (0.36)	-0.08	-4.91				
	IFT Females (n=6)	1.47 (0.41)	1.42 (0.37)	-0.05	-3.40				
	CERT Females (n=9)	1.49 (0.37)	1.62 (0.22)	0.13	8.72				
VI EI (arbitrary units)	IFT Males (n=7)	38.4 (21.0)	35.6 (18.5)	-2.8	-7.3	0.99	0.75	0.65	0.90
	CERT Males (n=5)	33.5 (16.1)	35.1 (7.2)	1.6	4.8				
	IFT Females (n=6)	40.4 (10.5)	44.2 (29.2)	3.8	9.4				
	CERT Females (n=9)	48.4 (10.9)	41.8 (9.1)	-6.6	-13.6				

Note: Mean (*SD*). [1] Significantly different than baseline at week 12 in all groups combined. [2]Significant time x group interaction. [3]Significant time x sex interaction. [4]Data reported are untransformed; statistical analyses for intra-muscular fat are based on Log10 transformed values. BMI = Body Mass Index, CSA = cross-sectional area, EI = echogenicity, Group = main effect for diet group, I = time x group interaction, LBM = Lean Body Mass, RF = rectus femoris, S = time x sex interaction, Time = main effect for time, VI = vastus intermedius. pQCT was conducted on n = 31. Ultrasound was conducted on n = 27

Dietary intake analysis

Participant dietary intake data measured before and during the intervention are summarised in Table 5.3. There was a main effect for time for overall average absolute daily energy intake in kJ [F(1, 30) = 9.61, *p*=0.006], relative energy intake in kJ/kg [F(1, 30) = 6.34, *p*=0.008], relative protein intake in g/kg [F(1, 30) = 22.72, *p*<0.001], relative carbohydrate intake in g/kg [F(1, 30) = 14.34, *p*=0.001] and relative fat in g/kg [F(1, 30) = 16.67, *p*<0.001], with significant reductions in energy (both absolute and relative), relative carbohydrate and fat intake identified across all groups, and a significant increase in relative protein intake. A significant time x sex interaction was found for relative energy intake (kj/kg) [F(1, 30) = 5.64, *p*=0.03] and relative carbohydrate intake (g/kg) [F(1,30) = 7.56, *p*=0.01], with females demonstrating greater reductions in energy and carbohydrate intake compared to males during the intervention period.

Table 5.3. The effects of 12 weeks of IFT and CERT on energy and macronutrient intake in male and female participants.

Diet variable	Group	Baseline	During Intervention[3]	Δ	Δ (%)	*P* (group)	*P* (time)	*P* (I)	*P* (S)
Average daily energy intake (kJ)	IFT Males (n=9)	8134 (1993)	7678 (909)	-456	-5.6	0.32	0.006[1]	0.69	0.09
	CERT Males (n=8)	8222 (1930)	7981 (836)	-241	-2.9				
	IFT Females (n=8)	7041 (957)	5538 (646)	-1503	-21.3				
	CERT Females (n=9)	7469 (1939)	6219 (680)	-1250	-16.7				
Energy (kJ/kg)	IFT Males (n=9)	94 (25)	91 (12)	-3	-3.2	0.43	0.008[1]	0.69	0.03[2]
	CERT Males (n=8)	95 (27)	94 (10)	-1	-1.1				
	IFT Females (n=8)	100 (21)	79 (9)	-21	-21.0				
	CERT Females (n=9)	106 (32)	88 (13)	-18	-17.0				
Protein (g/kg)	IFT Males (n=9)	1.28 (0.52)	1.50 (0.22)	0.22	17.19	0.80	<0.001[1]	0.48	0.46
	CERT Males (n=8)	1.08 (0.33)	1.53 (0.27)	0.45	41.67				
	IFT Females (n=8)	1.05 (0.15)	1.31 (0.13)	0.26	24.76				
	CERT Females (n=9)	1.18 (0.38)	1.42 (0.15)	0.24	20.34				
Carbohydrate (g/kg)	IFT Males (n=9)	2.21 (0.86)	2.07 (0.59)	-0.14	-6.33	0.31	0.001[1]	0.51	0.01[2]
	CERT Males (n=8)	2.36 (0.71)	2.28 (0.42)	-0.08	-3.39				
	IFT Females (n=8)	2.46 (0.92)	1.61 (0.39)	-0.85	-34.55				
	CERT Females (n=9)	2.56 (0.66)	1.94 (0.36)	-0.62	-24.22				
Fat (g/kg)	IFT Males (n=9)	0.87 (0.34)	0.76 (0.16)	-0.11	-12.64	0.61	<0.001[1]	0.56	0.34
	CERT Males (n=8)	0.91 (0.33)	0.74 (0.16)	-0.17	-18.68				
	IFT Females (n=8)	1.05 (0.24)	0.73 (0.07)	-0.32	-30.48				
	CERT Females (n=9)	0.99 (0.33)	0.79 (0.17)	-0.20	-20.20				

Note: Mean (*SD*). [1] Significantly different than baseline at week 12 in all groups combined. [2]Significant time x sex interaction. [3]Average of week 1, 6 and 12 intakes. Group = main effect for diet group, I = time x group interaction, S = time x sex interaction, Time = main effect for time.

Fasting versus non-fasting days in IFT participants

Differences in dietary energy and protein intake on fasting and non-fasting days for the IFT group are presented in Table 5.4. Energy intake on fasting days for females was significantly lower in week 12 compared to week 1 (p=0.009). No other significant differences between time points on fasting or non-fasting days were found.

Table 5.4. Dietary intake for fasting and non-fasting days for IFT male and female participants.

Diet variable	Time	IFT Males Non-Fast Days (n = 9)	IFT Males Fast Days (n = 9)	IFT Females Non-Fast Days (n = 8)	IFT Females Fast Days (n = 8)
Average daily Energy Intake (kJ)	Week 1	9596 (1028)	2456 (136)	7185 (1041)	1957 (344)
	Week 6	9682 (1426)	2502 (431)	6758 (856)	1951 (260)
	Week 12	9380 (1712)	2511 (0)	6530 (1075)	1686 (292)[1]
	Overall average[2]	9619 (1058)	2490 (175)	6824 (734)	1865 (276)
Energy (kJ/kg)	Week 1	113 (14)	29 (4)	100 (19)	27 (4)
	Week 6	116 (22)	30 (5)	97 (16)	28 (4)
	Week 12	114 (24)	31 (4)	94 (14)	24 (5)
	Overall average[2]	114 (17)	29.62 (4.03)	97 (13)	26 (4)
Protein (g/kg)	Week 1	1.62 (0.40)	1.07 (0 15)	1.54 (0.19)	0 98 (0.12)
	Week 6	1.70 (0.39)	1.08 (0 21)	1.45 (0.28)	0 99 (0.16)
	Week 12	1.68 (0.41)	1.14 (0 16)	1.30 (0.21)	0 94 (0.17)
	Overall average[2]	1.63 (0.35)	1.11 (0 15)	1.44 (0.16)	0 98 (0.13)

Note: Mean (*SD*). [1] Significantly different to week 1 values in specified group, $p<0.05$. [2]Average of week 1, 6 and 12 intakes.

Upper body and lower body 3RM strength and endurance volume analysis

Changes in bench press and leg press 3RM, and bench press and leg press endurance test volume are reported in Table 5.5. There were main effects for time for bench press 3RM [$F(1, 30) = 75.37$, $p<0.001$], bench press volume [$F(1, 30) = 46.06$, $p<0.001$], leg press 3RM [$F(1, 30) = 84.91$, $p<0.001$] and leg press volume [$F(1, 30) = 39.32$, $p<0.001$], with increases noted in each of these variables across the intervention. No other significant interactions or main effects were identified for strength or endurance variables.

Table 5.5. The effects of 12 weeks of IFT and CERT on strength variables in male and female participants.

Strength variable	Group	Baseline	Week 12	Δ	Δ (%)	*P* (group)	*P* (time)	*P* (I)	*P* (S)
Bench press 3RM (kg)	IFT Males (n=9)	58.0 (12.1)	61.7 (11.3)	3.7	6.4	0.67	<0.001[1]	0.35	0.07
	CERT Males (n=8)	57.0 (12.9)	61.1 (13.9)	4.1	7.2				
	IFT Females (n=8)	26.8 (4.9)	31.9 (3.0)	5.1	19.0				
	CERT Females (n=9)	23.9 (30.8)	30.8 (5.2)	6.9	28.9				
	IFT Males (n=9)	611 (197)	692 (190)	81	13.3	0.29	<0.001[1]	0.29	0.46

Bench press volume (70% 1RM) (kg)	CERT Males (n=8)	522 (128)	657 (103)	135	25.9				
	IFT Females (n=8)	283 (104)	406 (61)	123	43.5				
	CERT Females (n=9)	247 (66)	394 (64)	147	59.5				
Leg Press 3RM (kg)	IFT Males (n=9)	150.6 (37.1)	185.0 (44)	34.4	22.8	0.95	<0.001[1]	0.51	0.34
	CERT Males (n=8)	165.0 (47.9)	186.8 (48)	21.8	13.2				
	IFT Females (n=8)	68.8 (32.8)	101.8 (33)	33.0	48.0				
	CERT Females (n=9)	57.4 (22.8)	93.9 (22)	36.5	63.6				
Leg press volume (70% 1RM) (kg)	IFT Males (n=9)	1636 (782)	2356 (1154)	720	44.0	0.36	<0.001[1]	0.74	0.95
	CERT Males (n=8)	1468 (578)	1904 (345)	436	29.7				
	IFT Females (n=8)	759 (558)	1269 (840)	510	67.2				
	CERT Females (n=9)	582 (1248)	1248 (526)	666	114.4				

Note: Mean (*SD*). [1] Significantly different than baseline at week 12 in all groups combined. Group = main effect for diet group, I = time x group interaction, S = time x sex interaction, Time = main effect for time, 1RM = 1 repetition maximum, 3RM = 3 repetition maximum.

Correlations between variables and changes in LBM

Figure 5.1 shows the correlation between relative changes in body weight and body fat and LBM. There was a significant, moderate positive correlation between changes in LBM and weight in both groups overall (r = 0.63, *p*<0.001), indicating that as weight loss increased, gains in LBM reduced. When split by intervention group, this relationship was strengthened in the CERT group (r=0.86, *p*<0.001), while no significant relationship was seen for the IFT group (r = 0.23, *p*=0.38), with all but one IFT group participant increasing LBM regardless of percentage weight loss. Similarly, there was a moderate correlation between the percentage of overall fat mass lost with changes in LBM in the CERT group (r = 0.53, *p*=0.03), but not in the IFT group (r = -0.21, *p*=0.41). Change in LBM was negatively correlated with relative (kJ/kg) energy intake (r = -0.40, *p*=0.02) and carbohydrate (g/kg) intake (r = -0.36, *p*=0.03) and positively correlated with percent change in pQCT muscle area (r = 0.51, *p*=0.003). Relative change (percent) in bench press 3RM (r = 0.476, *p*=0.004) and leg press 3RM (r = 0.550, *p*=0.001) were also positively correlated with changes in LBM. No other measurements were found to have significant correlations with change in LBM.

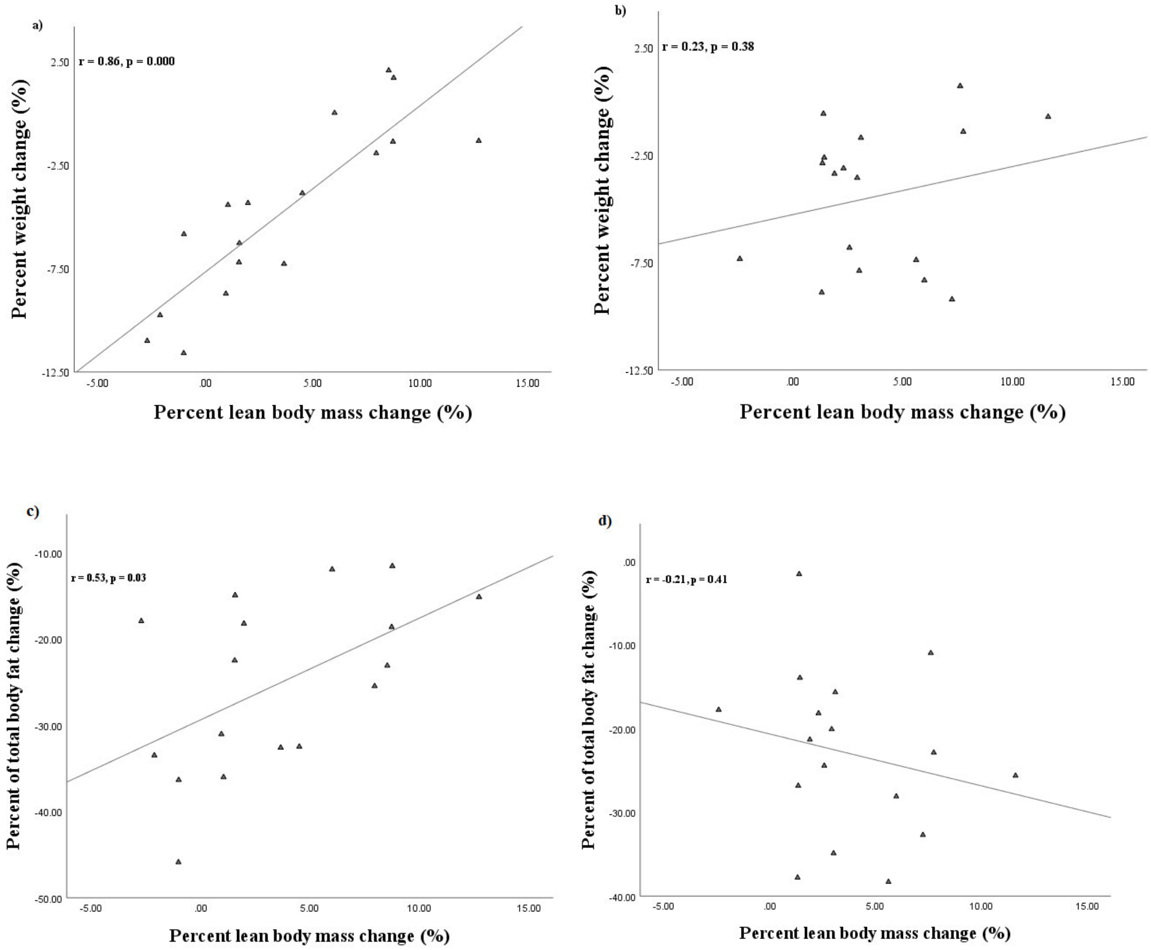


Figure 5.1. Bivariate correlations between changes in body weight and LBM in CERT groups (**a**) and IFT groups (**b**); and between changes in percentage of total fat mass lost and LBM in CERT (**c**) and IFT (**d**) groups.

Discussion

To the authors' knowledge, this is the first randomised trial to compare the effects of 5:2 intermittent fasting and continuous energy restriction on body composition and strength adaptations when concurrently undertaking resistance training. The findings suggest that despite different patterns of energy restriction, when overall dietary energy and protein intake are similar, both diets induce comparable increases in LBM and strength, and reductions in weight and fat over a 12 week period. In contrast, assessment of the mid-thigh CSA by pQCT demonstrated greater increases in the CERT group, which was supported by similar changes in RF thickness and CSA assessed by ultrasonography; albeit not significantly. Both groups however, experienced comparable reductions in intramuscular and subcutaneous fat, and improvements in muscle quality (EI). It was clear that there were sexual dimorphic differences in weight and LBM, with males demonstrating greater weight loss, but less gains in LBM, which may be a reflection of weight-loss-induced impairment of LBM accrual.

In the current study, both dietary intervention groups experienced significant reductions in body fat (mean -7.2 kg) and increases in LBM (mean +1.7 kg) suggesting weight lost was exclusively from fat. These observations were supported by localised changes in the thigh muscle with increases in RF thickness and CSA, and improvements in muscle quality as indicated by decreases in RF EI and reductions in intramuscular and subcutaneous fat measured by ultrasonography and pQCT respectively. The changes in pQCT muscle surface area were positively correlated with changes in whole body LBM ($r = 0.51$, $p=0.003$), suggesting muscular hypertrophy may have underpinned the increases in LBM. Taken together, these findings support the notion that muscle accrual can occur together with reductions in fat mass, especially when energy deficits are combined with resistance training and protein intakes above standard daily recommendations (19,151,152).

To the authors’ knowledge, there is only 1 other IF plus resistance training study that has resulted in statistically significant increases in LBM (102). In this study, trained women undertook a resistance training program combined with an 8 hour TRF diet (including $\geqslant$ 1.4

g/kg/day protein consumption), with or without supplementation of β-hydroxy β-methylbutyrate (HMB) (102). LBM was increased by an average of 1.2 kg and 0.9 kg in the HMB and non-HMB groups, respectively. However, despite a prescribed energy deficit, participants in this study gained weight, suggesting they were in apparent energy surplus. Conversely, the majority of other studies investigating IF (1 ADF and 3 TRF) with resistance training have demonstrated either a small loss, no change, or statistically non-significant growth of LBM (-0.4 kg to + 0.6 kg), accompanied by small to moderate reductions in weight (-1.0 kg to -3.3 kg) (25,101,150,153). Indeed, prior to this study, there appeared to be limited evidence that resistance training could induce significant accrual of LBM while undertaking an IF style diet. It is important to note that IF is a broad concept and that while ADF, TRF and 5:2 all fit under this umbrella, there are significant differences in how these diets are applied. For example, while TRF uses extended periods of fasting each day (16-20 hours), if an energy deficit is prescribed it is generally still prescribed on a daily basis. On the other hand, while ADF and 5:2 IF are similar in that they generally prescribe severe restrictions on fasting days and no restrictions on non-fasting days, the number of fasting days each week is significantly different. Therefore, it is difficult to make direct comparisons between these results and those of previous studies, even if the diets used are all broadly classified as IF.

Similarly, only a handful of CER studies have shown significant increases in LBM with concurrent reductions in weight. In two studies from the same group, Longland et al. (152) and Josse et al. (151) showed increases in LBM of 1.2 kg (1.6%) and 0.7kg (1.4%), with body weight losses of 5.9 kg (5.9%) and 4.3 kg (5.0%) respectively. Participants in the Longland et al. (152) study were required to consume either 1.2 g/kg/day or 2.4 g/kg/day of protein and undertake intense exercise 6 days/week, including 2 resistance training sessions (with the remainder aerobic exercise or plyometric weight circuits) for 4 weeks. Only those in the higher protein group gained, while those in the lower protein group maintained LBM. The authors attributed these results to a combination of intense exercise and high protein intake. Similarly, participants in the Josse et al. (151) study undertook 7 days of exercise each week, including 2 resistance training sessions (plus 7 days of aerobic exercise), with a lower protein intake of 1.3 g/kg/day. However, the duration of this study was much longer at 16 weeks. These results suggest when weight loss via CER is concentrated over a shorter period of time, higher protein intakes may be necessary for LBM accrual. Indeed, these results are congruent with ours, with a 1.4 g/kg/day protein intake sufficient to support accrual of LBM over a longer intervention period. There were a number of similarities between this study and those of Longland et al. (152) and Josse et al. (151). Namely that the majority of exercise sessions were supervised and that participants had frequent contact and feedback with regards to their diets (although it should be noted that participants in the Longland et al. (152) study also had all food provided for them). This may have helped promote high levels compliance to dietary and training protocols. The most notable difference between this study and theirs (apart from pattern of energy intake in the IFT group) was the frequency of exercise, with participants in this study exercising only 3 times per week. It is possible that a reduced frequency of aerobic training may have helped promote greater LBM accrual in this study. There is some evidence to suggest that performing both resistance and aerobic exercise together may result in a concurrent training effect, whereby LBM growth may be compromised, especially as frequency of endurance training increases (154). The practical implications of this may also be important, as training 3 days/week compared to 6-7 days/week may be more appealing/realistic for a significant portion of the general population.

Although both dietary groups experienced significant increases in LBM and reductions in weight and body fat, some notable differences between sexes in terms of the magnitude of change were observed. Female participants gained significantly more LBM on average compared to males (2.5 kg versus 0.9 kg, $p=0.003$), but experienced less weight loss (-2.2 kg versus -5.5 kg, $p=0.001$) and fat loss (-6.4 kg versus -8.0 kg, $p=0.18$), though the latter was non-significant. Conversely, no significant differences between sexes were evident in variables assessed by ultrasound and pQCT. A number of sex-specific factors may explain the disparity in weight and fat reduction between males and females. Participants in the CERT group were prescribed a relative energy deficit of 20% of total estimated energy expenditure, while in the IFT group this ranged from 20-23% due to the standardised nature of fasting days. Nonetheless, this would have led to a greater absolute energy deficit for males by ~600 kJ/day given their comparatively larger lean mass. While this amount may seem small, given that the reported energy deficit for both sexes was ~10% more than prescribed (~30%), the absolute difference between sexes was most likely greater. When combined with the fact that females may require a greater absolute energy deficit per unit of weight-loss (155), are more likely to under-report energy intake (156) and may be impacted by hormones that promote weight retention (157,158), it is not surprising that males experienced greater weight and fat loss in the current study.

The differences in total amounts (and therefore overall rate) of weight loss between sexes could also explain the greater LBM gains in females compared to males. Previous research has shown that slower rates of weight loss may be beneficial for LBM preservation, though this finding is not ubiquitous in the literature (159). Congruent with this, when all weight loss and LBM changes for both sexes were pooled together in the current study, there was a moderate, positive correlation between change in weight and change in LBM ($r = 0.63$, $p<0.001$). That is, as weight loss increased, LBM gains were reduced and potentially compromised. However, when split by diet, this relationship strengthened in the CERT group (r = 0.86, $p<0.001$), but weakened in the IFT group to be statistically non-significant (r = 0.23, $p=0.38$). This trend was also seen when comparing relative overall fat loss with changes in LBM. These findings may suggest that when paired with resistance training, the 5:2 style diet could be protective/promote gains in LBM compared with continuous energy restriction when greater amounts of weight loss occur.

Given that there were no differences in the reported overall intake of energy or any macronutrient between groups, it seems likely that the uncoupling of weight and LBM changes may have been driven by the differing patterns of consumption prescribed by the diets, i.e. 2 versus 7 days of energy restriction. One possible explanation for this, is the differing responses to anabolic stimuli that occur during energy restriction compared to energy balance. It has been shown that compared to energy balance, continuous, short-term energy restriction leads to a blunting of MPS in response to feeding (142), and in the anabolic hormonal response to resistance training (160). However, while these effects have been found over short time periods of continuous energy restriction (3-5 days), it is unclear how these responses might differ when periods of energy deficit are kept short (~24 hours) and interspersed with periods of energy sufficiency, which is the aim of the 5:2 diet. While the importance of acute hormonal and muscle protein synthetic response to stimuli with regards to longer-term LBM growth is not well understood (161,162) it is possible that over 12 weeks, small differences in anabolic response could accumulate and create meaningful differences in LBM growth in favour of a 5:2 style diet. However, this requires further investigation. Alternatively, the fact that all training sessions were completed on non-fasting days, when relative energy and substrate availability would have been higher in the IFT compared to the

CERT group could have impacted training-induced LBM adaptations. However, similar to the MPS and hormonal responses discussed above, it is currently unclear how these two factors might interact.

On the contrary, analysis of the local thigh musculature may not support the notion of superiority for 5:2 IF. Ultrasound assessment showed a greater increase in RF thickness in the CERT compared to the IFT group (CERT = + 11.2% versus IFT = +5.9%, p=0.05), with a similar trend for RF CSA (CERT = +19.2% versus IFT = +9.2%, p=0.07). Likewise, pQCT showed significantly greater increases in muscle area in the CERT group compared to IFT group (CERT = + 4.2% versus IFT = 0.0%, p=0.03). Disparity between groups observed at the whole LBM compared to local musculature level could be due to a number of reasons. Firstly, it is known that hypertrophy in response to resistance training does not occur uniformly throughout the body, with upper body more responsive than lower body muscle mass (163–165). It is likely that changes in upper body musculature between groups were missed by only assessing the thigh muscle via pQCT and ultrasound. In support of this, it was noted that when examining DXA-derived regional differences in LBM, the IFT group showed greater increases in the upper body compared to those in the CERT group, albeit marginally, while the opposite was true for the lower body (not reported). Secondly, the small, uneven sample size for the more sensitive assessments of muscle may have been insufficient to identify real differences between diet groups, especially given the known large inter-individual variability in adaptations to resistance training (166). Finally, it is important to note that muscle mass is only one component of DXA-derived LBM, measurement of which can be affected by changes in non-muscle lean tissue such as bone, water, connective tissue and even fat-free adipose tissue (167). Thus, while changes in DXA-derived LBM and the more sensitive methods of muscle assessment did not show complete agreement, differences may have been mediated by changes in other, non-muscle components of LBM.

Although there were similar trends in measures of hypertrophy as assessed by pQCT and ultrasound (in terms of group differences), there were some notable differences in the magnitude of change. As measured by ultrasound, average increases in this study were approximately 9% for RF thickness, and 18% for RF CSA. Change in thickness is roughly in-line with previous research, which has shown increases in overall quadricep thickness of around 5-9% in response to 12 week training programs (163,168). In terms of muscle surface area, the magnitude of growth when measured by pQCT was much smaller (mean +2.1%, p=0.10) than that seen in RF thickness or CSA. This discrepancy in magnitude is likely a result of differences in the two techniques. The pQCT scan area includes a cross section of the entire thigh, meaning that it is inclusive of all local muscles. Thus changes in the VI, and posterior, medial and lateral thigh muscles may have contributed to the smaller (and overall non-significant) changes in the pQCT scan.

Both ultrasound and pQCT generally showed improvements in measures of muscular fat infiltration. Ultrasound of the RF showed a reduction in EI (mean -4.6 AU). RF EI is utilised as a measure of muscle quality, with higher values representing greater levels of intramuscular fat. This measure may have clinical importance as smaller EI values have been associated with greater muscular strength in some populations, independent of muscle size (169). Similarly, the pQCT showed a reduction in intramuscular fat (mean -0.6 cm^2), lower levels of which have been shown to be associated with a reduced risk of type 2 diabetes (128), and greater levels with poorer strength (170). The change in intramuscular fat can be considered consistent with a reduction in RF EI and both indicate an improvement in muscle quality.

Despite the noted differences in body composition changes between sexes, both dietary groups and sexes demonstrated significant increases in upper and lower body muscle strength and endurance over the 12 week period. Improvements in 3RM for bench and leg press, and bench press and leg press endurance volume were approximately 17%, 45%, 49% and 97% respectively. Magnitude of strength change appeared to be in-line with previous research into untrained individuals for leg press (171), however changes in bench press 3RM were smaller than might be expected for a 12 week intervention (172). The differences between the observed results in these measures and past research could be due to the intentional exclusion of these two specific exercises (leg press and bench press) in the resistance training program in this study (for logistical and safety purposes). Early increases in strength in untrained individuals are often attributed to neuromuscular adaptations, however, over the course of 8-12 weeks muscular hypertrophy may also play a significant role (165). Indeed, the results showed that changes in bench press and leg press 3RM were both significantly and positively correlated with DXA-derived changes in whole body LBM ($r = 0.48$, p=0.004 and $r = 0.55$, p=0.001 respectively). This further emphasises the need to focus on growing or maintaining LBM during energy restriction, as it may help promote increases in physical strength and function.

While no time x sex interaction was found for any strength/endurance variable, when expressed as a percentage change from baseline, females appeared to show greater gains which is consistent with some (173,174), but not all (163,175) previous research. This can potentially be explained by greater increases in LBM in females, and lower initial levels of strength (176) both in absolute terms and relative to LBM. Indeed, while a moderate positive correlation was found between increases in LBM and increases in the 3RM for both bench press and leg press as noted above, females also showed greater strength increases in each of these lifts expressed relative to LBM. While previous research has shown that males tend to be stronger than females per unit of LBM (177), others have suggested that when previous levels of physical activity are similar, differences in strength are almost entirely related to muscle size (178,179). Although the study specifically excluded participants who had followed a structured resistance training program in the previous 6 months, it did not investigate levels of physical activity prior to this. Thus, while speculative, it is possible that males had greater familiarity with the types of exercises performed, or were generally more physically active prior to the study, which may have resulted in a reduced relative response in strength.

There were a number of strengths of the current study. Firstly, participants were supervised for the majority of their training sessions, allowing standardisation of form and effort. Secondly, frequent access to the study dietitian may have helped improve motivation and compliance. Thirdly, the study utilised non-invasive and reliable methods of assessment to measure changes in muscle size and quality alongside whole body LBM (pQCT and ultrasound), both of which have shown utility in clinical settings (169,180).

Our study was also subject to a number of limitations. Firstly, while frequent supervision and feedback for training and diet may have helped with study validity, this may not be practical for the general population. Secondly, although detailed dietary data was collected, the limitations of self-reported dietary intake are well known, and the ability to make strong inferences based on dietary data were limited. However, reported intake did reflect the aims of this study (i.e. increased intake on non-fasting days for the IFT compared to the CERT group). Thirdly, the study included recently untrained individuals only, and the results cannot be generalised to those with differing training histories. Fourthly, it is unclear how the provision of supplements to the IFT group on fasting days may have affected compliance, or whether the supplements themselves may have contributed to the results. Finally, as menstrual cycle details were not recorded for females, it is unclear whether this may have affected the results.

In summary, 5:2 intermittent fasting or continuous energy restriction combined with moderate protein intake and resistance training over 12 weeks led to comparable LBM gains, weight and body fat loss, and improvements in muscle strength, endurance and quality. However, there were clear sexual dimorphic differences in a number of these outcomes, and some disparities noted between measures of body composition and local muscle changes. Interestingly, there did appear to be some benefit to the 5:2 style energy restriction for LBM as total weight loss increased, however the reason for this is not immediately clear. Therefore future studies should investigate the longer-term impacts of 5:2 fasting in comparison to continuous energy restriction, but importantly, explore the relationship between transient changes in energy balance and protein turnover, weight loss and muscle mass accrual.

Chapter Six

Chapter Six: Effect of intervention on cardio-metabolic markers

6.1 Preface to chapter

The following chapter is written as an extended manuscript and aims to answer the second research question: are the two interventions comparable in terms of their effect on blood markers of cardio-metabolic health?

The data in the below paper was collected from 31 participants, as blood draw failed on 3. All data presented pertains to these 31 individuals, and as such, baseline characteristics and changes in weight and body composition are slightly different to that seen in the previous chapter.

6.2 The effects of the intervention on cardio-metabolic markers

The impact of 12 weeks of resistance training with 5:2 intermittent fasting or continuous energy restriction on cardio-metabolic health

Abstract

Background: Excess adiposity is associated with increased risk factors for cardiovascular disease and type 2 diabetes, regardless of body mass index. Energy restricted diets can lead to both body weight and fat loss, reducing risk of disease. While continuous energy restriction is commonly used to achieve this, intermittent fasting is gaining popularity.

Objectives: To explore the impact of both 5:2 intermittent fasting and continuous energy restriction on blood markers of cardio-metabolic health.

Methods: Thirty-one individuals underwent 12 weeks of either 5:2 intermittent fasting (5 days of euenergetic consumption, 2 non-consecutive days of ~30% energy requirements) or continuous energy restriction (consumption of ~80% of energy requirements daily) while undertaking a resistance training program 3 times per week. Fasted serum samples were taken at baseline and post-intervention, and blood lipids, glucose, insulin and high-sensitivity CRP were analysed.

Results: 5:2 intermittent fasting led to greater reductions in total cholesterol and LDL-cholesterol than continuous energy restriction. This was likely related to greater baseline values. There were no significant changes triglycerides, high-sensitivity CRP or markers of glucose metabolism.

Conclusions: In generally healthy individuals, 5:2 intermittent fasting led to greater reductions in total and LDL-cholesterol, but had no significant effect on markers of glucose metabolism or inflammation.

Introduction

Overweight and obesity, conditions generally accompanied by excessive body fat, are a common and growing health concern around the world (181). Excess adiposity is associated with insulin resistance (182), derangement of blood lipids (54) and increases in systemic inflammatory markers (183) that may increase the risk of a number of health conditions. While excess adiposity is often thought of in the context of overweight and obesity, the associated risks are not unique to individuals who fit into these categories. Indeed, individuals who are of normal weight but have a high body fat percentage often show some degree of metabolic dysregulation (3), that may increase the risk of conditions such as cardiovascular disease (51) or promote the development of insulin resistance (52) and type 2 diabetes (184). Thus, regardless of BMI, those with excess adiposity may benefit from the reduction in weight and body fat that generally accompanies energy restricted diets (185,186).

There are various dietary strategies that individuals can utilise to restrict their energy intake, including more traditional methods such as continuous energy restriction (CER), and others such as intermittent fasting (IF), which has gained recent popularity. While the benefits of these diets are typically linked to weight loss induced by energy restriction, there has been suggestion that IF may provide metabolic health benefits that are independent of weight loss (187), and thus may be superior to CER in this regard. In particular, the 5:2 style of IF has been shown to reduce fasting insulin levels when compared to CER (93,95), though the differences may be of a magnitude that lacks clinical significance (188). Conversely, there appears to be little difference in the effects of CER and IF on blood lipids (92,94,95) or markers of inflammation (92,93).

While there is substantial evidence that markers of metabolic and cardiovascular health are improved through energy restricted diets (both IF and CER), the efficacy of these interventions may be enhanced when utilised in combination with various modes of exercise (153,189). Resistance training may have the added benefit of helping to preserve lean body mass (LBM) and increase strength, which are important considerations for overall physical function and quality of life, especially in ageing (190). While there are a number of studies that have investigated the effects of IF combined with resistance training on cardio-metabolic markers (25,102,153), none to date have utilised 5:2 IF. Furthermore, none have included a CER comparison group where both groups experienced similar amounts of weight loss. Thus, the purpose of this study is to report changes in cardio-metabolic blood markers in individuals with excess adiposity undertaking 12 weeks of resistance training combined with either 5:2 IF or an isoenergetic CER diet.

Methods

Detailed study methods are presented in Chapter 4. The methods for the statistical analysis relating to the results presented in this chapter are presented below.

Statistics

All results are presented as mean (±SD). Normality of variables was assessed utilising the Shapiro-Wilk test and visual inspection of Q-Q plots. Assumptions of normality were violated for hsCRP, triglycerides, insulin and HOMA-IR. Insulin and HOMA-IR were log10 transformed to achieve normality, however normality could not be achieved for hsCRP and triglycerides. Three outlying hsCRP values (>10.0 mg/L) were removed as these were likely to be due to acute illness. The Wilcoxon signed-rank test was used to analyse differences in non-normal variables from baseline to post intervention. For normally distributed variables, linear mixed models were used to test for main effects of time, group and sex, and time x group, time x sex, group x sex and time x group x sex interactions. All model assumptions for linear mixed modelling were verified for all analyses. Individual changes are also represented graphically for each variable. Baseline data was analysed for differences between groups using independent t-tests and Mann Whitney U tests for normal and non-normal data respectively. All analyses were conducted using SPSS version 25. A *p*-value of <0.05 was considered significant for all tests.

Results

Baseline characteristics for participants are reported in Table 6.1. A total of 34 participants completed the intervention (IFT = 17, CERT = 17), however only 31 successfully had blood taken (IFT = 15, CERT = 16). Blood draws were attempted and failed on 3 participants. Baseline characteristics of completers (n=31) who had blood samples analysed are presented in Table 6.1. Significant differences were found between groups for TC only which was significantly higher in the IFT group than the CERT group. There were no other differences between groups at baseline.

Table 6.1. Baseline characteristics

Baseline variables	IFT Males (n = 8)	CERT Males (n = 8)	IFT Females (n = 7)	CERT Females (n = 8)	*p*-value (group)
Age (years)	26.0 (6.1)	23.1 (2.6)	24.4 (2.9)	23.6 (5.1)	0.24
Height (cm)	181.2 (0 1)	178.6 (0.1)	162.0 (0.1)	163.8 (0.1)	0.80

Weight (kg)	89.1 (12 1)	88.0 (11.5)	71.9 (11.6)	71 5 (11.2)	0.95
BMI (kg/m^2)	27.1 (2.8)	27.6 (2.4)	27.3 (2.6)	26.6 (3.6)	0.82
Body Fat Percentage (%)	29.9 (6.2)	30.4 (3.7)	42.8 (4.1)	41 9 (6.7)	0.76
hsCRP (mg/L)	0.94 (0.64)	2.96 (4.63)	1.84 (1.74)	3.68 (3.87)	0.12[2]
TC (mmol/L)	4.5 (0.80)	4.18 (0.60)	5.17 (1.02)	4.18 (0.75)	0.0371[1]
Triglycerides (mmol/L)	0.94 (0.26)	1.20 (0.51)	1.01 (.36)	0.90 (0.36)	0.86[2]
HDL-C (mmol/L)	1.35 (0.47)	1.15 (0.32)	1.83 (0.46)	1.57 (0.43)	0.22
LDL-C (mmol/L)	2.71 (0.63)	2.49 (0.60)	2.86 (0.78)	2.19 (0.45)	0.05
Glucose (mmol/L)	4.88 (0.33)	4.90 (0.35)	4.84 (0.42)	4.76 (0.52)	0.84
Insulin (mU/L)[3]	8.55 (4.54)	10.26 (6.22)	10.40 (2.73)	11.89 (7.69)	0.59
HOMA-IR[3]	1.88 (1.07)	2.29 (1.60)	2.27 (0.75)	2.61 (2.02)	0.64

Note: Mean (*SD*). [1]Significantly different between IFT and CERT. [2]Data from Mann-Whitney U test. [3]Values presented are non-transformed, all analysis conducted on Log10 transformed values to achieve normality. BMI = body mass index, HDL-C = high density lipoprotein-cholesterol, hsCRP = high-sensitivity C-reactive protein, HOMA-IR = homeostatic model assessment of insulin resistance, LBM = lean body mass, LDL-C = low density lipoprotein-cholesterol, TC = total serum cholesterol.

Changes in BMI, body weight and body fat percentage

Changes in BMI, body weight and body fat percentage are presented in table 6.2. There was a main effect for time for BMI [F(1, 27) = 54.6, *p*=<0.0001], weight [F(1, 27) = 59.4, *p*<0.0001] and body fat percentage [F(1, 27) = 180.6, *p*<0.0001] indicating decreases in each variable in all groups over time. Further, there was a significant time x sex interaction for BMI [F(1, 27) = 8.8, *p*=0.006] and weight [F(1, 27) = 12.6, *p*=0.001] indicating that males reduced BMI and weight to a greater degree than females.

Table 6.2. Changes in BMI, body weight and body fat percentage over 12 weeks of IFT or CERT combined with resistance training in males and females.

Body composition variable	Group	Baseline	Post Intervention	Δ	Δ (%)	*P* (group)	*P* (time)	*P* (I)	*P* (S)
BMI (kg/m^2)	IFT Males (n=8)	27.1 (2.8)	25.7 (2.2)	-1.4	-5.2	0.79	<0.0001[1]	0 38	0.006[2]
	CERT Males (n=8)	27.6 (2.4)	25.5 (2.7)	-2.1	-7.6				
	IFT Females (n=7)	27.3 (2.6)	26.6 (2.6)	-0.7	-2.6				
	CERT Females (n=8)	26.6 (3.6)	25.9 (3.1)	-0.7	-2.6				
Weight (kg)	IFT Males (n=8)	89.1 (12.1)	84.6 (10.7)	-4.5	-5.1	0.76	<0.0001[1]	0 34	0.001[2]
	CERT Males (n=8)	88.0 (11.5)	81.7 (12.1)	-6.3	-7.2				
	IFT Females (n=7)	71.9 (11.6)	70.0 (10.5)	-1.9	-2.6				
	CERT Females (n=8)	71.5 (11.2)	69.4 (9.2)	-2.1	-2.9				

Body fat percentage (%)	IFT Males (n=8)	29.9 (6.2)	23.9 (4.3)	-6.0	-20.1	0.80	<0.0001[1]	0 56	0.60
	CERT Males (n=8)	30.4 (3.7)	22.8 (4.3)	-7.6	-25.0				
	IFT Females (n=7)	42.8 (4.1)	36.3 (2.4)	-6.5	-15.2				
	CERT Females (n=8)	41.9 (6.7)	35.9 (7.2)	-6.0	-14.3				

Note: Mean (SD). [1]Significantly different than baseline at post-intervention in all groups combined. [2]Significant time x sex interaction. Group = main effect for diet group, I = time x group interaction, S = time x sex interaction, Time = main effect for time.

Changes in blood lipids, glucose, insulin, HOMA-IR and hsCRP are shown in Table 6.3. A main effect of time was found indicating reductions in TC [F(1, 27) = 20.18, *p*=0.0001], LDL-C [F(1, 27) = 22.1, *p*=0.0001] and HDL-C [F(1, 27) = 4.26, *p*=0.049]. There was a significant time x group interaction for TC [F(1, 27) = 5.76, *p*=0.024] and LDL-C cholesterol [F(1, 27) = 5.78, *p*=0.023], with those in the IFT groups experiencing a reduction compared to CERT groups. For HDL-C, there was also a significant time x sex interaction [F(1, 27) = 12.79, *p*=0.001] indicating females experienced a reduction compared to males. No other main effects or interactions were found for any other variable.

Table 6.3. Changes in blood markers of cardio-metabolic health over 12 weeks of IFT or CERT combined with resistance training in males and females.

Diet variable	Group	Baseline	Post Intervention	Δ	Δ (%)	*P* (group)	*P* (time)	*P* (I)	*P* (S)
TC (mmol/L)	IFT Males (n=8)	4.49 (.79)	4.03 (.68)	-0.46	-10.2	0.10	0.0001[1]	0.024[2]	0.06
	CERT Males (n=7)	4.18 (.60)	3.79 (.44)	-0.39	-9.3				
	IFT Females (n=8)	5.17 (1.02)	4.36 (.76)	-0.81	-15.7				
	CERT Females (n=8)	4.18 (.75)	4.18 (.82)	0.0	0.0				
LDL-C (mmol/L)	IFT Males (n=8)	2.71 (.63)	2.23 (.51)	-0.48	-17.7	0.17	0.0001[1]	0.023[2]	0.10
	CERT Males (n=7)	2.49 (.60)	2.10 (.48)	-0.39	-15.7				
	IFT Females (n=8)	2.86 (.78)	2.30 (.58)	-0.56	-19.6				
	CERT Females (n=8)	2.19 (.45)	2.24 (.47)	0.05	2.3				
HDL-C (mmol/L)	IFT Males (n=8)	1.35 (.47)	1.38 (.44)	0.03	2.2	0.23	0.049[1]	0.08	0.001[3]
	CERT Males (n=7)	1.15 (.32)	1.21 (.35)	0.06	5.2				
	IFT Females (n=8)	1.83 (.46)	1.58 (.38)	-0.25	-13.7				
	CERT Females (n=8)	1.57 (.43)	1.50 (.37)	-0.07	-4.6				
Glucose (mmol/L)	IFT Males (n=8)	4.88 (.33)	4.95 (.28)	0.07	1.4	0.91	0.09	0.55	0.35
	CERT Males (n=7)	4.90 (.35)	4.94 (.27)	0.04	0.8				
	IFT Females (n=8)	4.84 (.42)	4.93 (.32)	0.09	1.9				
	CERT Females (n=8)	4.76 (.52)	5.05 (.41)	0.29	6.1				
	IFT Males (n=8)	8.55 (4.54)	7.38 (2.13)	-1.17	-13.7	0.95	0.21	0.29	0.79

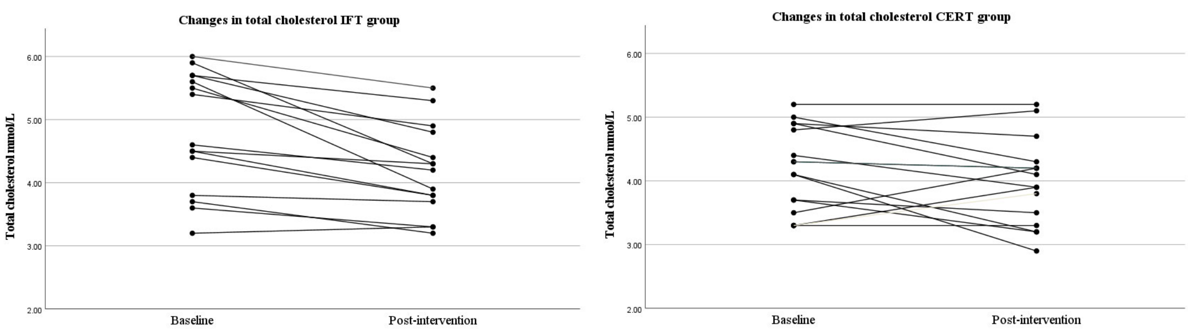
Log10(Insulin) (mU/L)	CERT Males (n=7)	10.26 (6.22)	8.56 (5.59)	-1.70	-16.6				
	IFT Females (n=8)	10.40 (2.73)	10.61 (2.43)	0.21	2.0				
	CERT Females (n=8)	11.89 (7.69)	8.85 (4.59)	-3.04	-25.6				
Log10(HOMA_IR)	IFT Males (n=8)	1.88 (1.07)	1.62 (.49)	-0.26	-13.8	0.96	0.36	0.36	0.73
	CERT Males (n=7)	2.29 (1.60)	1.93 (1.38)	-0.36	-15.7				
	IFT Females (n=8)	2.27 (.75)	2.32 (.51)	0.05	2.2				
	CERT Females (n=8)	2.61 (2.02)	2.05 (1.31)	-0.56	-21.5				
Non-normal variables	Group	Baseline	Post Intervention	Δ	Δ (%)	Signed rank Wilcoxon test (paired samples)			
						Group/sex		Diet group	
hsCRP (mg/L)	IFT Males (n=8)	0.94 (.64)	0.59 (.35)	-0.35	-37.2	Z = -1.13, *p* = 0.26		Z = 0.00, *p* = 1.00	
	CERT Males (n=6)	1.41 (1.63)	1.07 (1.26)	-0.34	-24.1	Z = -0.94, *p* = 0.35		Z = -1.73, *p* = 0.08	
	IFT Females (n=7)	1.95 (1.88)	2.45 (2.14)	0.50	25.6	Z = -1.16, *p* = 0.25			
	CERT Females (n=7)	2.43 (1.72)	1.36 (0.91)	-1.07	-44.0	Z = -1.36, *p* = 0.17			
Triglycerides (mmol/L)	IFT Males (n=8)	0.94 (.26)	0.93 (.35)	-0.01	-1.1	Z = -0.53, *p* = 0.60		Z = -0.36, *p* = 0.71	
	CERT Males (n=7)	1.20 (.51)	1.06 (.47)	-0.14	-11.7	Z = -1.02, *p* = 0.31		Z = -0.35, *p* = 0.73	
	IFT Females (n=8)	1.01 (.36)	1.04 (.44)	0.03	3.0	Z = -0.18, *p* = 0.85			
	CERT Females (n=8)	0.90 (.36)	0.98 (.37)	0.08	8.9	Z = -0.84, *p* = 0.40			

Note: Mean (*SD*). [1] Significantly different than baseline at week 12 in all groups combined. [2]Significant time x diet group interaction. [3]Significant time x sex interaction. Group = main effect for diet group, I = time x group interaction, S = time x sex interaction, Time = main effect for time.

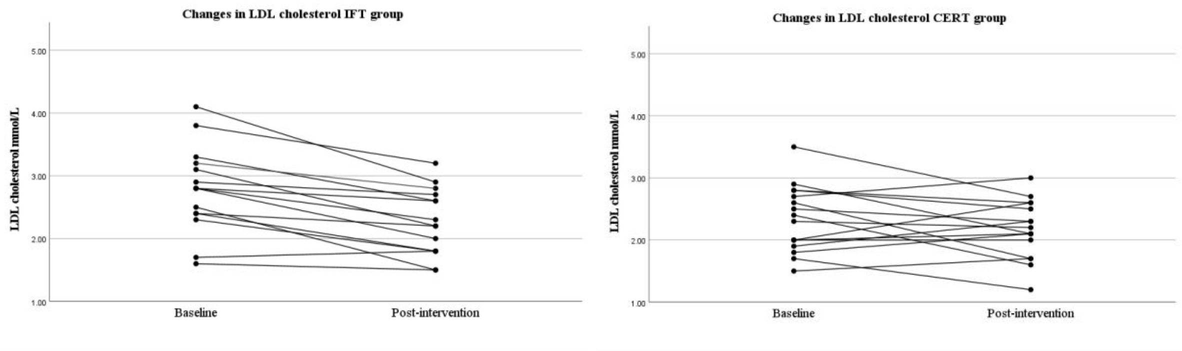
Individual changes for participants where main effects were noted (TC, LDL-C and HDL-C) are shown in Figure 6.1.

Figure 6.1. Individual changes from baseline to post intervention in a) TC; b) LDL-C and; c) HDL-C split by dietary group.

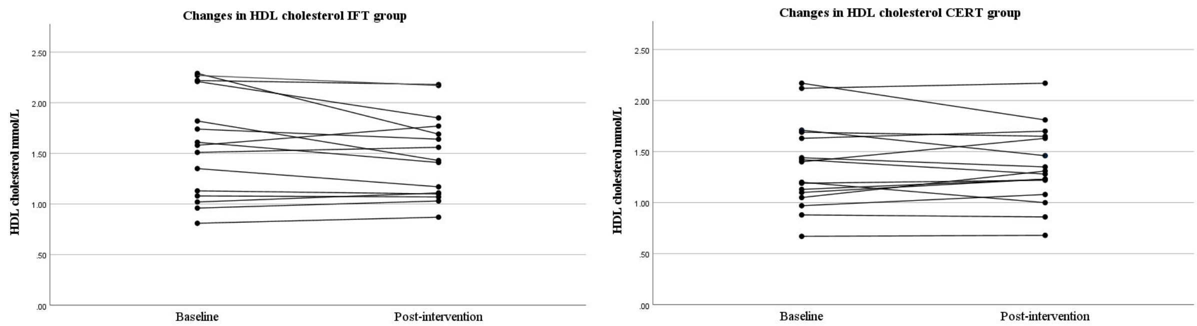
a)



b)



c)



Discussion

The main findings from this study are that there was a main effect for time indicating reductions in TC, LDL-C and HDL-C, but no significant changes in measures of insulin resistance, hsCRP or triglycerides. However, there appeared to be differences to the pattern of changes between males and females. Males in both dietary groups experienced similar mean changes (in direction and mostly in magnitude) for all measures, while responses differed between females depending on their dietary intervention. It is likely that these differing responses by sex are due to disparity in the magnitude of weight loss, and baseline values of each marker measured.

Our results showed that there was a significantly greater decrease in TC and LDL-C in the IFT group compared to the CERT group. These results are in contrast to a recent systematic review and meta-analysis that showed that CER may result in greater improvements in TC (although similar changes in LDL-C) when compared to IF (191). However, there appeared to be a notable disparity between sexes in how diet affected cholesterol levels. While males in both dietary groups experienced reductions in TC and LDL-C, the same was only seen in IFT females. This discrepancy can likely be explained by differing baseline levels and degree of weight loss. While it has been reported that TC levels and changes in weight have a positive, linear relationship (186), generally effects are proposed to occur when weight loss is >5% of initial body weight (192). Indeed, in this study, males lost an average of 6.4% of their body weight, and experienced a reduction in TC and LDL-C regardless of group. Conversely, average weight reduction in females was only 2.9%. Notwithstanding, females in the IFT group did experience a reduction in TC and LDL-C, however this was likely mediated by higher baseline values. In their review, Meng et al. (191) showed via sub-analysis that individuals with starting cholesterol levels over ~5.1 mmol/L (i.e. values similar to those seen in female IFT participants in this study) reported greater reductions in TC. Thus, while weight loss may not have of been of a magnitude large enough to induce changes in TC and LDL-C in all females in this study, it is possible that dietary changes and exercise were sufficient to produce changes in individuals with higher baseline values. The same pattern was noted with HDL-C; males in both groups experienced a small increase, while females in both groups experienced a slight reduction, with females in the IFT group having higher baseline values and experiencing a greater drop in HDL-C than those in the CERT group.

We found similar trends in measures of insulin resistance to those seen for blood lipids in that males in both dietary groups showed a reduction in HOMA-IR. However, in contrast to blood lipid results, only females in the CERT group showed a reduction. Thus, females in the IFT group were the only participants not to experience a mean improvement in HOMA-IR.

Interestingly, this is congruent with some previous research showing increases in fasting insulin in overweight females following IF. In their study, Hutchison et al. (69) utilised 2 different intermittent ADF protocols; 1 designed to induce weight loss and 1 designed not to induce weight loss via increased intake on non-fasting days. While the weight loss group reduced their body weight by 5.4 kg and experienced a reduction in fasting insulin levels, the non-weight loss group lost a similar amount of weight to the female IFT participants observed in this study (2.7kg), and experienced an increase in fasting insulin at the end of the 8 week intervention. This was found when measurements were taken after a feeding day, and authors speculated this to be due to the overfeeding prescribed in this group on these days. In contrast, in this study, researchers took blood samples after the end of the intervention period (within 1 week), when all participants had likely resumed their habitual diets (though this was not measured). Interestingly, IFT females were also the only sub-group to experience a mean increase in hsCRP, which is proposed to be a downstream marker of insulin resistance (193) and may add weight to the findings, however, once outliers were removed this increase was no longer significant. Together, these results may indicate that in the absence of more substantial weight loss, IF may undermine improvements in insulin sensitivity seen with energy restricted diets.

Notwithstanding the above discussion, the form in which IF is implemented may also be of importance. It has been suggested that longer periods of complete fasting may be required to induce benefits to metabolic health, and that this may only occur in the 5:2 IF style when fasting days are consecutive (96). Indeed previous studies have shown that 5:2 IF can result in improvements in insulin sensitivity over and above that seen in CER comparator groups when fasting days are consecutive (93,95). Conversely, studies separating fasting days have shown no benefit over CER for markers of blood glucose control (94,105,108). As discussed by Meng et al. (191), this is consistent with theories that the beneficial metabolic effects of IF may occur during extended periods of fasting (12-36 hours) sufficient to cause depletion of hepatic glycogen stores, and a switch to the metabolism of lipid-derived substrates (24). While it was stipulated that participants in the IFT group should consume their food between 12pm and 6pm each day, when consumption actually occurred over this period was not recorder, nor when the last or first feeding occurred on the previous or subsequent days respectively. Thus, it is possible that fasting periods in the IFT group were not sufficient to induce beneficial changes in insulin sensitivity seen in some past 5:2 IF studies. It should also be noted that individuals showed marked variability in their responses to the interventions (see figure 6.1), with participants in both groups containing individuals who experienced improvement and deterioration in their HOMA IR (range IFT = -1.9 to +1.11, CERT = -3.9 to + 2.9). Thus there may also be other underlying factors that affected change that this study was not able to elucidate.

In conclusion, while males in both dietary groups experienced beneficial changes in blood lipid profile and markers of insulin resistance and inflammation, results for females were disparate depending on dietary group. This may be due to differences in overall weight loss experienced and baseline values. However, the sample size was small, and data presented were a secondary outcome from a larger study investigating changes in body composition. Furthermore, while the study utilised generally healthy individuals, baseline values and changes over the intervention period varied considerably, which may have confounded the interpretation. The study also did not include diet only comparator groups, thus it is unclear whether diet and resistance training impacted values differently. Future research should aim to further differentiate the effects of IF style independent of weight loss.

Chapter Seven

Chapter Seven: Effect of intervention on ratings of compliance, mood, hunger and energy levels

7.1 Preface to chapter

The following chapter is written as an extended manuscript and aims to answer the third research question: Does either dietary intervention lead to different levels of compliance, or ratings of mood, hunger, cravings or energy levels?

7.2 The effects of the intervention on ratings of compliance, mood, hunger and energy levels

Self-reported dietary compliance is similar between individuals undertaking 5:2 intermittent fasting and continuous energy restriction over a 12 week period.

Abstract

Background: Intermittent fasting is often promoted as a version of energy restriction that may result in greater compliance, due to the fact that restriction is only needed during concentrated time periods. An individual's ability to comply may be related to a number of underlying factors, including hunger, cravings and mood.

Objectives: To compare rates of compliance between a 5:2 fasting and continuous energy restriction diet over a 12 week intervention period. A further objective was to investigate the effects of each diet on ratings of hunger, mood and energy levels.

Methods: Thirty-four participants engaged in either a 5:2 intermittent fasting (IFT) or continuous energy restricted diet (CERT) for 12 weeks. The average energy restriction for both groups was ~20%. Those in the 5:2 intermittent fasting group restricted their consumption to ~30% of energy requirements twice per week on non-consecutive days utilising meal replacement shakes, soups and vegetables. The continuous energy restriction group restricted their energy to ~80% of requirements daily. Each day participants completed a survey with 17 questions pertaining to compliance, hunger, mood and energy levels using a 10 point Likert scale.

Results: Ratings of compliance were similarly high in both groups (mean compliance, IFT = 8.4 ± 1.0, CERT = 8.0 ± 1.3, p = 0.28), while hunger and cravings were low from the beginning of the study in both groups and reduced further over time. The majority of participants (n = 29) indicated that they would continue with their dietary intervention at the end of the 12 weeks, albeit in a modified form.

Conclusions: Both diets resulted in high levels of compliance, which may have been in part due to the support provided throughout the intervention. While participants indicated a willingness to continue with their diets, these diets may require customisation to the individual in order for them to be maintained beyond 12 weeks.

Introduction

Recent data reveals that approximately 40% of the adult population around the world has attempted to lose weight, generally through dieting (194). However, the success of any weight loss diet is dependent on an individual's ability to comply (195), and dietary compliance over the long-term is notoriously poor (7). While there are a number of factors associated with poor dietary compliance (7), increased hunger and cravings caused by restrictive dieting play a part in undermining the success of long-term weight loss diets. Indeed the degree of hunger experienced during weight loss has been shown to be a predictor of future weight regain (196), and thus should be an important consideration for practitioners when monitoring individuals on restrictive diets. However, overeating and suboptimal food choices are not always a result of physiological hunger or food cravings, with mood and emotional state also being a key driver in some individuals, particularly amongst regular dieters (197). It is important to consider how any diet targeting weight loss affects all of these variables, as they may help determine likelihood of compliance and thereby long-term success. This knowledge may also assist health practitioners to identify and overcome potential road-blocks for individuals when prescribing energy restricted diets.

Continuous energy restriction [CER (restricting energy on a daily basis)] is often recommended as best practice for weight loss (198). However, recently intermittent fasting [IF (interspersing severe energy restriction with ad libitum or euenergetic consumption)] has gained popularity as an alternative method of energy restriction. It has been suggested that the intermittent nature of restriction in IF may make it easier to comply with in comparison to CER, given dietary restriction is not constant (199). However, to date, compliance has shown to be generally similar between these diets in studies that have compared them (200), while similar changes in hunger, appetite and mood have also been noted (93,201). Despite this, there are numerous variations of IF, ranging from alternate day fasting (ADF) to time-restricted feeding (TRF), and each may affect these outcomes in different ways. One popular variety of IF is 5:2 fasting, where dieters restrict energy intake only 2 days per week. Few previous studies have compared the effects of CER and 5:2 IF on measures of compliance, hunger, cravings, energy levels or mood (93,107). While these studies found similar outcomes for both diets, measures were taken either at monthly increments (93) or at baseline and end of intervention only (107), which may not be able to identify more transient patterns of change over the course of these diets. Furthermore, neither of these studies investigated the intentions of participants to continue with their diets post-intervention, which may be an important indicator of their feasibility in the long-term. Thus, the purpose of this study was to investigate and compare the effects of a CER and 5:2 IF diet on compliance, hunger, cravings, mood and energy levels over a 12 week period utilising daily surveys to produce continuous data. Additionally, participants' intentions to continue with their prescribed diet were explored. This report utilises data that was collected as a secondary outcome in a study exploring the impact of either 5:2 IF or CER diets paired with resistance training on body composition.

Methods

Detailed study methods are presented in Chapter 4. The methods for the statistical analysis relating to the results presented in this chapter are presented below.

Statistical analysis

Cronbach's alpha was used to quantify the internal consistency of survey questions within the same measure (hunger, cravings, energy levels and mood). Alpha coefficients above 0.7 were regarded as high internal consistency. Linear mixed models were used to analyse survey responses for main effects of time, group and sex, and time x group, time x sex, group x sex and time x group x sex interactions using the mean of each week's responses. Furthermore, this same method was utilised for analysing differences on fasting and non-fasting (fed) days for the IFT group, with effect of group replaced by condition (fasted or fed). Subsequently, pairwise comparisons using Sidak's adjustment for multiple comparisons were utilised to determine in which weeks mean values were significantly different. All model assumptions for linear mixed modelling were verified for all analyses. The means for weeks 1, 6 and 12 are presented as well as continuous weekly data, which are presented graphically using mean values and 95% confidence intervals. For missing data, average values were imputed using all other data from each individual's survey. Thus, the missing values were representative of the mean value for every other day for that participant. Post-intervention questionnaire responses were analysed using independent t-tests or a descriptive analysis of responses. All analyses were conducted using SPSS version 25. A *p*-value of <0.05 was considered significant for all tests.

Results

Baseline characteristics have been reported previously in Chapter 5. Briefly, a total of 34 participants completed the intervention, with 10 withdrawing during the study prior to completion (IFT = 5, CERT = 5). Reasons for non-completion were inability to commit to the exercise sessions (n = 3), inability to commit to the diet (n = 2, CERT = 1, IFT = 1), or unrelated medical issues and relocation (n = 5). Baseline characteristics for participants can be seen in Table 7.1. There were no significant differences found between groups in baseline characteristics.

Table 7.1. Baseline participant characteristics.

Baseline variables	IFT Males (n = 9)	CERT Males (n = 8)	IFT Females (n = 8)	CERT Females (n = 9)	*p*-value[1]
Age (years)	25.2 (6.2)	23 1 (2.6)	24.3 (2.9)	23.2 (4.9)	0.31
Height (cm)	181.1 (0 1)	178.6 (0.1)	161.6 (0.1)	164.2 (0.1)	0.82
Weight (kg)	87.3 (12 5)	88.0 (11.5)	71.9 (10.7)	72.1 (10.6)	0.92
BMI (kg/m^2)	26.6 (3.0)	27.6 (2.4)	27.5 (2.5)	26.7 (3.4)	0.93
Body Fat Percentage (%)	29.4 (6.0)	30.4 (3.7)	43.0 (3.9)	42.2 (6.3)	0.76

Note: Mean (*SD*). [1]P-values reported are for independent t-tests between intervention groups. BMI = body mass index.

Table 7.2. Cronbach's alpha values for survey question groups.

	Cronbach's alpha*
Hunger	0.875
Cravings	0.746
Energy levels	0.724
Mood	0.912

**Based on week 1 responses*

Cronbach's alpha indicated that there was a high level of internal consistency for questions grouped in the hunger, cravings, energy levels and mood scores.

Table 7.3. The effects of 12 weeks of IFT and CERT on mean ratings of hunger, cravings, energy levels, mood and compliance.

Survey Variable	Group	Week 1	Week 6	Week 12	Δ	Δ (%)	*P* (group)	*P* (time)	*P* (I)	*P* (S)
Hunger (max. 40)	IFT Males (n=9)	15.6 (2.7)	14.7 (2.5)	12.8 (3.8)	-2.8	-17.9	0.88	0.01[1]	0.18	0.03[2]
	CERT Males (n=8)	14.7 (4.4)	12.3 (3.8)	12 1 (5.9)	-2.6	-17.7				
	IFT Females (n=8)	12.2 (3.3)	11.2 (2.3)	10 1 (2.2)	-2.1	-17.2				
	CERT Females (n=9)	15.1 (5.0)	11.9 (5.4)	13.6 (3.7)	-1.5	-9.9				
Cravings (max. 40)	IFT Males (n=9)	14.0 (4.6)	10.3 (4.6)	10 9 (4.9)	-3.1	-22.1	0.82	<0.001[3]	0.96	0.22
	CERT Males (n=8)	10.3 (6.1)	8.8 (6.9)	9.4 (5.5)	-0.9	-8.7				
	IFT Females (n=8)	11.3 (4.5)	8.3 (4.2)	6.5 (4.4)	-4.8	-42.5				
	CERT Females (n=9)	12.9 (5.9)	9.0 (4.8)	8.9 (3.0)	-4.0	-31.0				
Energy levels (max. 40)	IFT Males (n=9)	21.1 (4.2)	22.9 (4.0)	21.4 (4.7)	0.3	1.4	0.49	0.91	0.39	0.17
	CERT Males (n=8)	21.7 (5.7)	21.0 (5.8)	21 2 (7.6)	-0.5	-2.3				
	IFT Females (n=8)	20.0 (5.6)	21.0 (5.3)	20.7 (7.2)	0.7	3.5				
	CERT Females (n=9)	18.6 (4.9)	17.1 (3.5)	17 3 (4.7)	-1.3	-7.0				
Mood (max. 40)	IFT Males (n=9)	31.0 (4.3)	30.1 (3.5)	29.6 (4.0)	-1.4	-4.5	0.046[4]	0.24	0.06	0.07
	CERT Males (n=8)	27.7 (3.0)	26.0 (3.9)	27.7 (2.7)	0.0	0.0				
	IFT Females (n=8)	28.9 (2.7)	28.4 (3.2)	28 3 (3.8)	-0.6	-2.1				
	CERT Females (n=9)	23.8 (6.8)	23.4 (7.2)	23.4 (7.5)	-0.4	-1.7				
Compliance (max. 10)	IFT Males (n=9)	8.4 (0.7)	8.7 (0.9)	8.9 (0.7)	0.5	6.0	0.28	0.29	0.88	0.09
	CERT Males (n=8)	8.5 (1.0)	8.1 (1.3)	8.3 (1.3)	-0.2	-2.4				
	IFT Females (n=8)	8.1 (1.2)	8.0 (0.8)	8.1 (1.4)	0.0	0.0				
	CERT Females (n=9)	7.6 (1.1)	7.6 (1.4)	7.7 (1.2)	0.1	1.3				

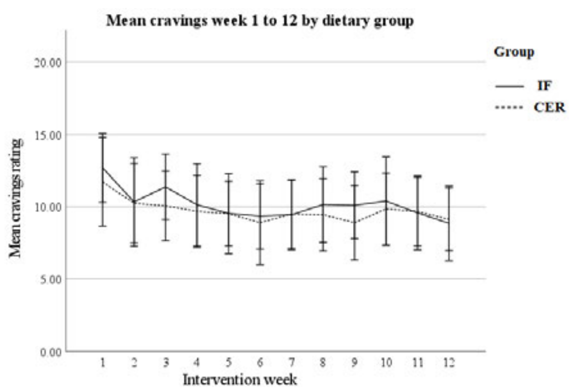
Note: Mean (*SD*). Δ represents change from week 1 to week 12. [1]Significantly different from week 1 in week 6 and 12 in all groups combined. [2]Significant main effect for sex. [3]Significantly different from week 1 in weeks 4-12 in all groups combined. [4]Significant main effect for diet group. Group = main effect for diet group, I = time x group interaction, S = main effect for sex, Time = main effect for time.

Ratings for hunger, cravings, energy levels, mood and compliance were collected for each day of the study over the 12 weeks intervention. As an example, results from weeks 1, 6 and 12 are reported in Table 7.3. A main effect for time was found for hunger [$F(1, 330) = 2.27$, $p=0.011$] and cravings [$F(1, 330) = 3.90$, $p<0.001$], indicating a decrease in both over the intervention period for all groups. Pairwise comparisons revealed that reported hunger was significantly lower than week 1 in weeks 6 and 12 only (but not weeks 2-5 and 7-11), while significant reductions in reported cravings compared to week 1 were found from weeks 4 to 12. Additionally, there was a main effect for diet on mood [F(1, 30) = 4.34, *p*=0.046], reflecting a lower reported mood score in the CERT group compared to the IFT group. Finally, there was a main effect for sex for hunger [F(1, 30) = 5.03, *p*=0.033] with males reporting greater levels of hunger than females. There were no other main effects or any interactions found.

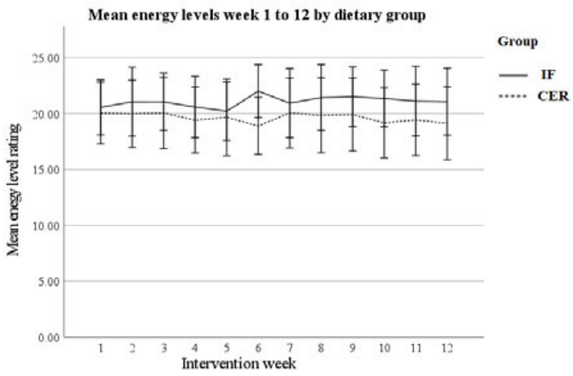
Weekly mean reported scores over the 12 weeks for hunger, cravings, energy levels, mood and compliance are shown graphically by diet group in Figure 7.1.

Figure 7.1. Weekly mean ratings for hunger (a), cravings (b), energy levels (c), mood (d) and compliance (e) over 12 weeks for CERT and IFT participants.

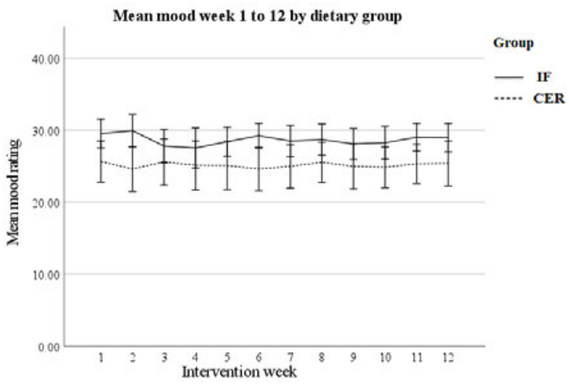
(a)



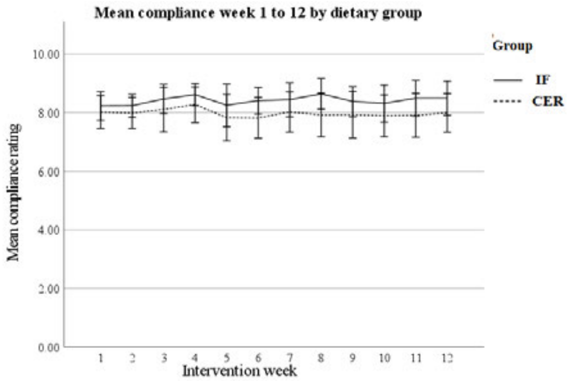
(b)



(c)



(d)



(e)

Table 7.4. Mean ratings for hunger, cravings, energy levels, mood and compliance separated by fasting and fed days in IFT participants over 12 weeks.

Survey Variable	Group	Week 1	Week 6	Week 12	Δ	Δ (%)	*P* (C)	*P* (time)	*P* (CS)	*P* (S)
Hunger (max. 40)	IFT Males (n=9) fed	11 2 (3.2)	9.7 (3.6)	9.2 (3.5)	-2.0	-17.9	<0.001[1]	0.88	0.013[2]	0.006[3]
	IFT Males (n=9) fasted	26 5 (5.2)	27.0 (6.3)	26.7 (7.6)	0.2	0.8				
	IFT Females (n=8) fed	7.7 (3.1)	6.9 (4.0)	7.6 (2.7)	-0.1	-1.3				
	IFT Females (n=9) fasted	23.6 (6.0)	21.8 (6.4)	22.4 (5.3)	-0.8	-3.4				
Cravings (max 40.)	IFT Males (n=9) fed	11 9 (4.6)	7.0 (4 9)	8.2 (4.8)	-3.7	-31.1	<0.001[1]	0.46	0.71	0.08
	IFT Males (n=9) fasted	19 1 (5.4)	18.6 (5.3)	21.8 (6.7)	2.7	14 1				
	IFT Females (n=8) fed	7.8 (4.7)	4.6 (3 1)	4.5 (3.6)	-3.3	-42.3				
	IFT Females (n=9) fasted	19 9 (7.1)	17.4 (8.7)	17.6 (10.6)	-2.3	-11.6				
	IFT Males (n=9) fed	21.8 (4.7)	23.2 (4.6)	21.8 (4.8)	0.0	0.0	<0.001[1]	0 38	0.09	0.75

Energy levels (max.	IFT Males (n=9) fasted	19 3 (5.4)	22.2 (5.0)	19.4 (7.4)	0.1	0.5				
	IFT Females (n=8) fed	20 9 (6.7)	21.6 (5.5)	20.8 (7.2)	-0.1	-0.5				
	IFT Females (n=9) fasted	17.8 (3.7)	19.6 (5.8)	20.4 (7.9)	2.6	14.6				
Mood (max. 40)	IFT Males (n=9) fed	30.7 (4.7)	30.4 (3.7)	30.0 (3.8)	-0.7	-2.3	<0.001[1]	0.021[4]	0.01[2]	0.21
	IFT Males (n=9) fasted	28 5 (6.7)	29.1 (4.2)	28.0 (4.8)	-0.5	-1.8				
	IFT Females (n=8) fed	30.0 (2.8)	29.1 (3.2)	28.4 (3.8)	-1.6	-5.3				
	IFT Females (n=9) fasted	26.4 (5.9)	26.5 (4.4)	27.4 (5.7)	1.0	3.8				
Compliance (max. 10)	IFT Males (n=9) fed	8.1 (0.7)	8.6 (0 9)	8.7 (0.9)	0.6	7.4	<0.001[1]	0 29	0.25	0.08
	IFT Males (n=9) fasted	9.1 (1.1)	9.1 (1 2)	9.5 (0.6)	0.4	4.4				
	IFT Females (n=8) fed	7.9 (1.4)	7.9 (0.6)	8.0 (1.2)	0.1	1.3				
	IFT Females (n=9) fasted	8.6 (1.2)	8.4 (1 9)	8.3 (2.3)	-0.3	-3.5				

Note: Mean (*SD*). [1] Significant main effect for condition (fasted/fed). [2]Significant condition x sex interaction. [3]Significant main effect for sex. [4]Significantly different between week 2 and 4 only in all groups combined. C = main effect for condition (fasted/fed), CS = condition x sex interaction, S = main effect for sex, Time = main effect for time.

Ratings for hunger, cravings, energy levels, mood and compliance for the IFT group split by sex and condition (fasting days versus fed days) are presented in Table 7.4. There was a main effect for condition for hunger [F(1, 345) = 1289.38, *p*<0.001], cravings [F(1, 345) = 615.33, p<0.001], energy levels [F(1, 345) = 27.30, *p*<0.001], mood [F(1, 345) = 70.09, *p*<0.001] and compliance[F(1, 345) = 57.69, *p*<0.001], indicating lower scores for hunger, cravings and compliance, and higher scores for energy levels and mood on fed versus fasting days. Furthermore, there was a main effect for sex for hunger [F(1, 15) = 10.15, *p*=0.006] indicating lower overall hunger ratings in females than males (including both fasting and fed days), and a significant condition x sex effect indicating lower hunger on fasting days in IFT females over the 12 weeks compared to males, with no difference noted on fed days [F(1, 345) = 6.17, *p*=0.013]. A significant main effect for time was found for mood [F(1, 345) = 2.09, *p*=0.02], indicating a reduction in mood ratings, however post-hoc pair-wise analysis revealed this was only significantly different between weeks 2 and 4. Finally, there was a condition x sex interaction for mood [F(1, 345) = 6.66, *p*=0.01] indicating that mood rating was significantly lower on fasting compared to fed days in females only.

Table 7.5. Post-intervention questionnaire responses to the dietary intervention in IFT and CERT participants.

How easy was it to comply with your diet? (0 = not easy at all 10 = extremely easy)	Mean score (SD)	*P*-value
IFT	6.6 (1.8)	0.38
CER	7.1 (1.3)	
Would you have preferred to have been in the other diet group?	**Yes**	**No**
IFT	4	13
CER	3	14
Will you continue with your diet after the intervention period has finished?	**Yes**	**No**
IFT	14*	3
CERT	15*	2

**Only 1 participant in the IFT group and 2 in the CERT group indicated they would continue with their diet as prescribed, the remainder from each group noted they would modify it in some way.*

Responses to the post-intervention questionnaire are presented in Table 7.5. There was no difference between how easy each group found it to comply with their allocated diet. Only 4 participants in the IFT group and 3 in the CERT group reported that they would have preferred to have been in the other dietary group. Additionally while 14 participants in the IFT group and 15 in the CERT group indicated they would continue with their diet after the intervention period, only 3 indicated they would keep their diet the same as it had been prescribed. Common modifications that participants intended to make to their diets were lowering protein intake (n = 4), or in the IFT group specifically, reducing number of fasting days (n = 5) or changing intake to solid food or no intake at all on fasting days (n = 5). The most common difficulty noted by participants was complying with their diet during social encounters (IFT = 13, CERT = 14).

Discussion

The main findings of the present study are that individuals in both dietary groups reported similarly high levels of compliance and low levels of hunger and cravings, with reported hunger and craving levels also reducing in both groups over time. However, there was a main effect for diet group on measures of mood, indicating a lower mood state was reported by the CERT group. Those in the IFT group reported greater compliance on fasting days compared to fed days. However, fasting also led to increased ratings of hunger and cravings, and reductions in energy level and mood, though the latter was only significant in females. Finally, the majority of participants displayed an intention to continue with their respective diets, albeit generally in modified forms, emphasising that flexibility may be required when considering the long-term feasibility of different energy restricted diets.

We found similarly high rates of reported compliance in both the 5:2 IF and CER diet groups (mean compliance, IFT = 8.4 ± 1.0, CERT = 8.0 ± 1.3, $p = 0.28$). These results are comparable to those of previous studies that have compared 5:2 IF and CER, which have reported compliance rates in the range of 56-97% over 3 months, with no differences between diets (94,95,105,201). The one exception to this is Harvie et al. (93), who reported completion of ~75% of fasting days in their 5:2 group after 3 months, compared with participants in the CER group who met their prescribed 25% energy deficit on only 39% of days. It should be noted that while previous research has generally utilised dietary records, or the number of days participants reported fasting to measure compliance, this study utilised a daily, subjective rating. The authors believe that this is of importance, as not only are dietary records notoriously inaccurate, individuals may be more likely to comply with protocols on days when they are required to record their food intake. Furthermore, assessing compliance as a binary measure may be dismissive of the impact of 'non-compliant' days that are nonetheless energy restricted.

There were a number of factors that may have promoted high levels of dietary compliance in both groups. Firstly, the provision of supplements on fasting days may have helped promote the greater level of compliance seen on fasted versus fed days in the IFT group (202). Secondly, as the study dietitian supervised each resistance training session, they had regular contact with participants. Frequency of contact with a dietitian has been reported to improve the rate of weight loss in dieting individuals likely by promoting compliance (203). Thirdly, each participant received a meal plan customised to their preferences, based on their self-reported baseline diet. Additionally, they were able to add or remove any items from the meal plan that they wished, so long as they continued to meet their energy and protein targets across the day, and they were taught how this could be done. Indeed, a number of studies have shown that dietary compliance is greater when individuals are asked to consume a diet that is similar to their habitual diet (204,205). Lastly, participants were asked to self-record their dietary intake 3 times across the course of the study, however many decided to track their intake continuously throughout the 12 week intervention. Self-monitoring dietary intake has been consistently shown to improve compliance to weight loss regimens (206). Importantly, these strategies are largely consistent with previous studies that have compared 5:2 IF and CER. Indeed, all previous studies that have compared these two diets (and to the author's knowledge, all 5:2 IF studies) have provided regular dietary counselling or motivational support, while also providing meal plans or food portion lists for participants. These findings add further weight to the notion that individuals show high levels of compliance to either diet over the medium term when combined with adequate professional support.

Participants in the current study also reported relatively low levels of hunger and cravings. While it is often proposed that hunger may be a limiting factor in the success of restrictive diets, especially with IF (108), participants in this study reported a low level of overall hunger, and a further reduction in hunger ratings over the 12 weeks in both groups. This reduction is consistent with some (93,94,105,201,207), but not all (27,108) studies comparing IF and CER. Similarly, cravings reported by the participants in this study started low and reduced in tandem with hunger. Food cravings can drive consumption and have been previously linked to a higher BMI (208). While weight loss has been associated with an increase in cravings in some studies (209), in-line with the results observed in this study, a number have also reported a reduction (210,211). Rigid dietary structures and elimination of foods have been shown to result in increased cravings (212), and the low level of cravings reported by the participants in this study may have been a result of the flexible dieting structure provided. While the participants were required to meet energy and protein targets each day, they were permitted achieve this in whatever way they preferred (so long as they did not use dietary supplements, with the exception of the IFT group on fasting days). Thus, the flexibility allowed may have further aided compliance by minimising impact of cravings in both dietary groups.

The only statistically significant difference noted between the 2 diets was on ratings of mood, which were significantly lower in the CERT group. However, there was a group x time interaction indicating that mood trended downwards in the IFT group over the course of the 12 weeks (p = 0.06). Unfortunately, as baseline mood testing was not performed, it is difficult to know if the absolute differences seen in mood were due to the dietary intervention, or inherent in each group. Interestingly, poorer mood was consistently seen on fasting compared to fed days in the IFT group. Although this difference was small in magnitude, this suggests that the negative impacts of dietary restriction on mood may have been concentrated to fasting days, which is a key appeal of IF style diets compared to CER. This is an important consideration for practitioners to consider when recommending dietary interventions, given that an individual's capacity to self-regulate may be limited, and depleted more quickly in times of increased stress or mental load (213). Furthermore, given the tendency of individuals to adopt an 'all-or-nothing' approach to dieting, a dietary lapse can often lead to reactionary over-eating (214). CER provides greater opportunity for this to occur than IF does, which may be exacerbated if this type of dieting also causes poorer mood in general, or a consistent increase in stress as individuals are constantly having to restrict. However there is a paucity of long-term studies that include measures of compliance and mood, and thus this remains speculative.

At the end of the intervention period, participants rated the ease with which they were able to comply with their diet as 6.6 and 7.1 out of 10 in the IFT and CERT groups respectively. These values are lower than those reported at 3, 6 and 12 months in a previous study comparing 5:2 IF and CER (108). This may have been due to the nature of the prescribed diets, namely a higher recommended protein intake, and liquid meals on fasting days for the IFT group. Indeed nearly half of all participants in the current study reported difficulty meeting the protein targets (IFT = 5, CERT = 10), and half of those in the IFT group noted that they would have preferred solid foods instead of liquid-meals on fasting days. This was reflected further in the intentions of the participants at the end the intervention period. While a large majority indicated they would continue with their diet protocols post intervention, only a few intended to continue as originally prescribed. Common changes that participants planned to make were a reduction in protein regardless of dietary group, and a reduction in number of fasting days and/or the use of solid meals instead of liquid-meals on fasting days for those in the IFT group. Interestingly, in one of the few other studies to utilise liquid meals for their 5:2

IF fasting group, Harvie et al. (95) showed a much smaller proportion of 5:2 IF participants (58%) planned to continue with their diet after the 6 month intervention compared to the CER group (85%). Conversely Sundfør et al. (108), showed that participants in both 5:2 IF (utilising consumption of solid-food on fasting days) and CER diets had comparably strong intentions to continue with their diet after 12 months. Thus, while both diets appear to be tolerable, flexibility and customisation with regards to how energy deficits are implemented may be important for long-term compliance. It should also be noted that the vast majority of participants from both groups (CERT = 14, IFT = 13) reported difficulty complying with their diet during social situations, with 1 participant in the IFT group also reporting avoiding going out on fasting days. This could also have implications for long-term compliance and quality of life, and further emphasises the need for interventions that allow some flexibility.

The main strength of this study was the continuous nature of the data collection. However, this was also the main limitation, as response rates decreased by the end of the 12 weeks, and there was likely to be a habitual nature to how participants answered the questionnaire. Furthermore, while the included questions had been previously validated, they had been validated as visual analogue scales, not Likert scales as used in this study. Finally, amount of weight loss, which has been shown to potentially impact changes in hunger and cravings (136), was not controlled for in the analysis (though was comparable between groups as reported in Chapter 5). Notwithstanding, the results indicate that in the short to medium term, individuals display similarly high levels of compliance and low levels of hunger and cravings when undertaking a CER or 5:2 IF style diet with appropriate support and guidance. Thus both may be feasible options for healthcare practitioners when assisting individuals aiming to lose weight.

Chapter Eight

Chapter Eight: Discussion and future directions

8.1 Preface to chapter

The research documented in the preceding chapters successfully addressed the aims and objectives set out in Chapter 3, specifically answering the following questions:

1. Are there differential effects on body composition, muscle size and quality or strength when undertaking a resistance training program combined with either 5:2 IF or CER when diets are matched for energy deficit and protein intake in individuals with excess body fat?

2. Are the two interventions comparable in terms of their effect on blood markers of cardio-metabolic health?

3. Does either dietary intervention lead to different levels of compliance, or ratings of mood, hunger, cravings or energy levels?

This chapter will discuss the major findings of this thesis, while also providing possible future directions for research.

8.2 Addressing the gaps in the literature

To date research investigating IF in conjunction with resistance training is relatively rare in the literature, with the exception of TRF. This is surprising given that one of the proposed benefits of IF is that it may improve outcomes for LBM. The lack of studies including resistance training may be due to differences in the populations typically studied. The majority of research investigating ADF and 5:2 fasting has typically studied overweight or obese individuals, whereas those investigating TRF tend to utilise leaner participants and the former also tend to include proportionately more females than males. Furthermore, traditionally aerobic exercise is associated with weight loss compared to resistance training, and resistance training continues to be commonly seen as a more masculine activity (215). Thus, given most non-TRF IF studies have included proportionately greater females who are typically overweight or obese, it is perhaps not surprising that aerobic training has been chosen as an exercise modality. Nonetheless, as noted in Chapter 2, there is in fact only one study prior to the one presented in this thesis that has utilised a form of IF other than TRF and combined it with resistance training (153) resulting in a large gap in the literature which requires further exploration.

The fact that resistance training interventions with IF are largely limited to TRF is a point of importance. While TRF fits under the IF umbrella, the way it is implemented is quite different from other methods such as alternate day or 5:2 fasting. Although TRF does involve extended periods of fasting, the fact that this generally occurs on a daily basis makes it more akin to CER in some ways. Many of the proposed benefits of IF are related to the fasting periods; increases in fat oxidation and ketones, hormonal changes etc. (24,147). However, it is possible that some of the advantages of IF may lie in the periods between fasts (216), when individuals are able to consume relatively greater amounts of energy compared to those following a CER style diet. It’s known that MPS is down-regulated during times of energy restriction (142,143). It’s also known that energy restriction results in greater perceived effort during exercise (217). If it is the periods of energy sufficiency where the advantage truly lies (if one exists), then the short feeding windows allowed in TRF may not be able to elucidate this. Further, ADF may go too far in the other direction, and the frequent fasting and constant breaking up of energy sufficiency may negate the benefits of feeding periods. Thus, 5:2 fasting could be seen as an intermediate approach, that might lead to weight loss while also allowing long periods of energy sufficiency. It should be noted, however, that this discussion largely pertains to the possible benefits on body composition, as the cardio-metabolic health benefits of IF are proposed to be more related to extended periods of fasting (24,96).

The choice of 5:2 fasting was also made as a way of potentially optimising compliance to the diet, and minimising the impact of hunger on compliance. If one of the proposed benefits of IF is that it may be easier to comply with due to the reduced frequency of restriction, it makes sense that increasing the number of fasting days might lead to proportionately greater difficulty complying with the diet. The difficulty of restrictive dieting may lie in the increases in hunger and appetite that occur, or the effects that restriction could potentially have on mood. While a number of studies investigating 5:2 IF have reported on measures of either compliance, hunger or mood compared to CER, only 1 has reported on all of these measures (93). The aforementioned study was slightly different in design to the one reported in this thesis, in that fasting days were recommended to be undertaken consecutively, whereas participants in this study were required to have at least 1 day separating fast days. Their study also utilised solid food on fasting days instead of liquid meal replacements. Thus, it was considered important to include and compare measures of compliance, hunger and mood in this study.

8.3 Major findings

The following sections detail and discuss the major findings from this research study as presented in Chapters 5-7.

8.3.1 Changes in body composition and strength

The outcomes of this study did not support the hypothesis proposed in Chapter 3 that resistance training would promote superior adaptations in body composition when combined with 5:2 IF compared to CER. In fact, changes in whole-body LBM, fat mass and body fat percentage were quite similar between groups. It was noted that there appeared to be a difference between the way males and females responded to the interventions, though as discussed in Chapter 5, this was likely due to differences in absolute energy deficits experienced. There was also comparable increases in strength, and reductions in markers of intramuscular fat in the thigh muscles as measured by both ultrasound and pQCT for both dietary groups. However, those in the CERT group tended to experience greater increases in the size of the thigh muscles compared to IFT. While this was unexpected, this could potentially be explained by differences in hypertrophy in the upper versus lower body, or by differences in the type of tissue that each assessment method measures (whole body lean tissue versus muscle only), as discussed.

On the surface then, it appeared that CER and 5:2 fasting were equivalent at promoting losses in body fat and gains in LBM. The fact that both interventions generally led to weight loss *and* LBM gains is interesting in and of itself. As discussed in the literature review chapter, weight loss is typically associated with some level of LBM loss. While many other studies have shown that LBM gains and weight loss can occur over the same time-period (19,151,152,218,219), these interventions commonly include greater exercise volumes and/or protein intakes than were prescribed in this study, although tend to have similarly moderate levels of energy restriction.

Despite such similarity in responses between groups, a more intriguing finding was the responses to each diet when considering magnitude of weight loss. It was found that there was a strong, positive correlation between amount of weight lost and changes in LBM only in the CERT group. That is, as individuals lost greater amounts of weight, they accrued less LBM, a relationship that was remarkably linear. Conversely, no such relationship was apparent in the IFT group. In fact, only 1 participant lost LBM in the IFT group (compared to 4 in the CERT group). Interestingly, this 1 participant appeared to be less compliant with dietary recommendations in terms of energy and protein intake on non-fasting days, and subjectively he also appeared less motivated to follow the diet than most others. This was reflected in his dietary records, with an average protein consumption of only 1.0 g/kg/day which was the lowest of any participant. He also reported the second lowest energy intake relative to estimated requirements on non-fasting days in the 5:2 group (though the data from the one participant who was lower may be dubious given she reported the largest relative energy deficit of any IFT participant but actually gained weight over the 12 weeks). While it was not considered appropriate to remove his data from the overall analysis, it is interesting to consider his apparent non-compliance given he was the only individual in the IFT group to lose LBM. Notwithstanding, the possibility that the 5:2 fasting style may have helped promote LBM gain regardless of magnitude of weight loss is intriguing. This finding needs to be considered cautiously, however, given the variability in weight loss and changes in LBM seen within groups, and the small sample size.

8.3.2 Changes in cardio-metabolic health markers

This study found generally favourable changes in fasting blood lipids in terms of a reduction in TC and LDL-C across groups. However, a small reduction in HDL-C was also observed with no changes in triglycerides. In contrast to the hypothesis stated in Chapter 3 that both groups would show similar changes in cardio-metabolic blood markers, there was a greater improvement in blood lipids (TC, LDL-C) in the IFT group than the CERT group, however it is likely that this was mediated by greater baseline values. Looking closely at the individual data revealed that there was large inter-individual variability, which is to be expected given that the study utilised generally healthy participants. Although participants were carrying excess adipose tissue, which has been associated with an increase in risk factors for cardiovascular disease, their blood lipid values were generally not deranged. Nonetheless, the study provided evidence that 5:2 IF combined with resistance training can help improve blood lipid levels in individuals with starting values that are at the higher end of normal. Whether this is greater than, or equal to the reduction seen with CER requires further investigation.

Conversely, the study found no difference between groups in terms of fasting glucose, insulin or HOMA-IR, and similarly no significant improvement in these measures overall. As discussed in Chapter 6, some previous 5:2 IF studies have found that this type of fasting offers benefits to glucose metabolism compared to CER. However, it has been suggested that this might be unique to interventions the utilise consecutive fasting days due to the longer fasting period (96). Interestingly, HOMA-IR did improve in all groups apart from IFT females consequent to reductions in fasting insulin (fasting glucose remained relatively stable, with each group experiencing a small increase). The fact that females in both dietary groups lost minimal amounts of weight compared to the males, suggests the possibility that improvements in females may have been independent of weight loss in the CER group only. While males in both groups had similar reductions in HOMA-IR, any negative impact of 5:2 IF on this measure may have been overcome by the effects of weight loss. Although this is speculative at best, there is some evidence to show that in the context of minimal weight loss, ADF can lead to impaired insulin sensitivity compared to CER and ADF that causes weight loss (69). Whilst ADF differs from 5:2 IF, given that the fasting days in this study were non-consecutive it may be comparable.

8.3.3 Compliance, hunger, cravings, energy levels and mood

In contrast to the hypothesis generated in Chapter 3, that 5:2 IF would result in higher ratings of compliance, energy levels and mood, and reduced hunger and cravings, minimal differences were found between intervention groups. Both reported high levels of compliance throughout the 12 weeks, while hunger and cravings were low from week 1 and reduced over time. The only difference noted was in terms of mood, with those in the CERT group reporting lower average mood ratings than those in the IFT group. As noted in Chapter 7, however, given that baseline testing was not undertaken, it is unclear if this difference was inherent in the groups, or caused by the intervention. Interestingly, although perhaps not surprisingly, ratings of hunger and cravings were higher on fasting days than on fed days for those in the IFT group, while ratings of mood and energy levels were lower. Despite this, levels of compliance were higher on fasting days. While overall mean ratings were generally similar between the CERT and IFT groups, this highlights the possibility that the negative effects of restriction could be limited to fasting days when undertaking IF. The implications of this for longer-term compliance are interesting. If the negative aspects of energy restriction can be experienced over short periods of time each week, will this be easier to stick to in the long-term?

Thus far, research suggests the answer to the above question is no. Reports that have compared IF (and indeed 5:2 IF) over longer periods with CER have found little difference in terms of compliance. They have also found little difference in terms of the intention of individuals to continue with their diet post-intervention period (108). This study observed similar results, in that there were comparable numbers of participants in each group who intended to continue with their diets after the 12 week intervention. It was also observed that most participants, when asked after the intervention, would not have chosen to be in the alternate intervention group. This suggests some level of tolerability for both diets. However, almost all participants also noted that if they continued with the intervention, they would alter it in some way. In the IFT group, this commonly including reducing the number of fasting days; switching from liquid meals to solid food on fasting days; and a few noted that they would reduce their intake on non-fasting days, indicating that they found it difficult to reach their energy and protein targets. In the CERT group, generally participants said that they would reduce their protein intake. Thus, while there was an indication that the principles of each diet are palatable long-term, they may need flexibility and customisation over time.

This is not a surprising finding, as there is no one-size-fits-all approach to energy restriction. It raises the possibility that long-term compliance could be aided by slight alterations to dietary interventions. Indeed one 5:2 IF study showed that even though compliance rates dropped over the course of 6 months from 70% after month 1, to 64% at month 6, these rates lifted to 78% and 73% if you included participants who were still undertaking 1 fasting day per week (95). Similarly, the high rates of compliance observed in the research study presented in this thesis compared to a number of other studies were likely to be, in part, related to the way which compliance was assessed. As discussed in Chapter 7, compliance in this study was measured using a rating out of 10, based on how close individuals felt they were to complying with their energy and protein intakes each day. Thus, the scores also included measurements of days that were somewhat, but not completely compliant, which is an important distinction, especially given the all-or-nothing mentality that many individuals have towards energy restriction.

Importantly, all of the above results were observed in individuals that had significant ongoing support. They were provided detailed meal plans (see Appendix 6 for an example), taught how to substitute foods in and out of their diets while continuing to meet prescribed energy and protein targets, and had bi-weekly contact with the dietitian through their supervised resistance training sessions. This is not dissimilar to previous interventions comparing 5:2 IF and CER. This raises an important consideration; that rates of compliance generally drop over time (although were maintained in this study) despite some support. Most individuals who try to lose weight do not do so with adequate professional support, thus raising the question of whether either of these diets would be feasible for the general public. In this regard, it is possible that IF may have an advantage, given that it is likely easier to implement without having in-depth food knowledge (simply by not eating for a certain period of time). This might be especially true given studies have shown that people tend not to compensate for fasting periods by eating more during non-fasting periods (220), thus individuals would solely have to concentrate on frequent fasting.

The importance of the way in which 5:2 fasting is implemented is also a question raised by this study. While compliance was high in the IFT group throughout, there were a number of challenges raised over the 12 weeks relating to consuming the supplements provided. The daily intake on fasting days for the IFT group was 1.5-2 protein shakes (with water) depending on sex, 1 high protein soup and 150g of raw or steamed vegetables. Despite this prescription, several participants reported challenges consuming the provided soup due to personal preferences and were therefore allowed to switch out the soup with an additional ½ a shake (which minimally impacted overall energy and protein intake on fasting days). However, despite an obvious preference for the shake over the soup generally, there was also feedback received about the protein shake itself (specifically taste and texture), and a number of individuals reported that they would not, or could not consume their second protein shake on some fasting days. Whilst the researchers tried to prevent this by providing potential participants with shakes to trial during screening and confirming that they would be willing to consume this on their fasting days for the full 12 weeks, as well as offering a variety of flavours, this was clearly not a foolproof method. In the post-intervention questionnaire, a number of participants did comment that they felt that fasting days would have been easier either consuming nothing at all, or consuming a solid food based diet. As noted in Chapter 7, the only other 5:2 IF study previously to utilise a mostly liquid diet on fasting days also showed a significant drop off in compliance over the course of the intervention (95). While compliance was similarly low in their CER comparison group, this does not discount the possibility that utilising liquid-based diets for IF may be less tolerable than whole foods.

8.4 Strengths

There were a number of strengths of this project. Firstly, it explored a variety of outcomes, including objective measures of body composition and cardio-metabolic health, and subjective measures of compliance, hunger, energy levels and mood. This allowed assessment of not only participants' physiological responses to the intervention, but also their ability to tolerate each intervention. This provided a rich data set that provides novel insight into how these two diets compare. Secondly, it utilised an exercise intervention that has been uncommon in all forms of IF apart from TRF, but is also known to promote maintenance or growth of LBM. Thirdly, it utilised a number of outcomes to measure change in body composition; including whole-body measures from DXA and local changes in the thigh musculature in terms of both size and quality from ultrasound and pQCT. This is especially novel and important as a number of studies have shown that muscle quality, as opposed to quantity may be related to physical function and strength, however these cannot be picked up on more blunt tools such as DXA. Fourthly, it utilised a continuous data set to show how compliance, hunger, energy levels and mood changed over-time in response to the intervention. Finally, the PhD candidate was involved in all aspects of the study, including in the measurement of all outcomes and given that he was also the study dietitian and strength and conditioning coach supervising training, all participants were provided with the same level of advice, and care throughout.

8.5 Limitations

There were a number of limitations with this research, and many of the strengths of the project were double edged. While it utilised multiple methods to quantify changes in body composition, it did not use the more sensitive methods to estimate changes in the upper body, which it appeared could have caused incongruence between the whole-body and local body composition data. Next, although the survey data was continuous, response rates varied, despite participants being reminded up to 5 times per week via text message to complete them. Thus for some individuals a considerable amount of data required imputation. Additionally, the fact that the PhD candidate was involved in all parts of the study, also meant that blinding of data collection and analysis was, for the most part, very difficult. While he tried to always maintain rigorous standards when collecting data, there is the possibility that this may have introduced bias to the results. Finally, and possibly most importantly, the sample size was small. While the number of participants is similar to many other studies in this field, it nonetheless reduces the ability to draw conclusive judgments from the data.

8.6 Future directions

There were a number of intriguing outcomes from this project that deserve further research. These include:

1. One of the more intriguing findings from this project was the relationship between amount of weight lost and changes in LBM. The fact that those in the 5:2 IFT group appeared to be able to gain greater amounts of LBM than the CERT group at increasing magnitudes of weight loss potentially has significant implications for individuals trying to lose more substantial amounts of weight. Future studies should aim to replicate these findings with larger numbers while controlling for weight loss. Of interest would be a study that utilises these methods to either induce weight loss of varying magnitudes, or that repeats body composition measures as weight loss increases in participants. A number of recent studies have utilised a percentage weight loss as a goal, rather than a specific time-frame. It would be worthwhile to assess individuals as they progress through their weight loss on each diet, for example outcomes might be re-assessed at 2%, 4%, 6%, 8% and 10% weight loss, to observe if 5:2 fasting is truly protective of LBM as weight loss increases.
2. There are a number of possible mechanisms that could potentially promote greater outcomes for LBM in 5:2 fasters compared to CER. One which has received limited focus to date, is how periods of energy sufficiency effect effort and volume during training sessions, and their impact on MPS in response to both feeding and resistance training. Future studies should aim to assess muscle protein synthetic response compared to baseline both in the short to medium term after following either a CER

or 5:2 diet. Of particular interest is the response in 5:2 fasters on feeding days in response to feeding and training.

3. Given the observed incongruences in the whole-body and local body compositional changes, future studies should ensure that sensitive methods of body composition analysis are used globally rather than in only one location. Some studies have utilised ultrasound to quantify changes in the biceps, for example, which may give an indication of upper body hypertrophy.
4. While a resistance training program was included in this study due to its ability to promote beneficial changes in LBM, the lack of a diet only control group meant that it was not possible to separate the effects of the dietary interventions from the effects of the exercise intervention. Future studies should aim to include diet only control groups, so that these effects can be illuminated.
5. It was also noted that all previous 5:2 IF studies appeared to have provided substantial support to participants in terms of meal plans, education and frequent motivational meetings and phone calls. As many individuals in the general population looking to lose weight do not engage appropriate professional support, it would be interesting to see how levels of compliance change when minimal support is provided. This is especially relevant given that promoters of IF consistently refer to the possibility that IF may be easier to comply with than a traditional CER type diet.
6. Participants in the current study, although reporting high levels of compliance and willingness to continue with their dietary interventions, did not seem to enjoy the liquid nature of the fasting day diets. It was noted that at least one previous study had utilised a similar protocol, and compliance rates were lower than what has generally

been reported. Thus, it would also be of interest to investigate whether a solid or liquid diet on fasting days is preferable.

8.7 Conclusion

This research presents novel findings revealing that, when paired with resistance training over a 12 week period, both 5:2 IF and CER result in comparable weight reduction and increases in LBM and strength. Intriguingly, there was evidence that 5:2 IF may have promoted greater LBM accrual as magnitude of weight loss increased compared to CER. Conversely, while both groups experienced increases in muscle size as assessed by ultrasound and pQCT, this appeared to be greater in the CERT group, an effect that could have been masked in DXA-derived LBM assessments by changes in non-muscle lean tissue. Notwithstanding, both groups showed similar improvements in muscle quality when measured via these sensitive assessment methods, both of which possess known clinical utility. Both interventions were also effective at improving levels of TC and LDL-C, but neither resulted in significant improvements in glucose metabolism. Reported levels of compliance were high throughout the 12 week intervention, while ratings of hunger and cravings were comparably low, indicating that both diets are well tolerated in the short-medium term and are likely to be practical for individuals seeking to undertake an energy restricted diet. While more research is necessary to fully illuminate differences between these styles of energy intake, this research is the first to show that when individuals undertake resistance training, 5:2 IF is a viable and comparable alternative to CER.

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Appendices

Appendix 1. Authorship declaration form for systematic literature review.

Authorship Agreement

This form and any supporting material must be retained to support authorship of publications.

AUTHORSHIP DECLARATION:

The criterion for 'authorship' is clearly defined in the Authorship guide published by the NHMRC, ARC and UA.

An author is someone who:

- has made a significant intellectual or scholarly contribution to research and its output, and
- agrees to be listed as an author.

A significant intellectual or scholarly contribution must include one, and should include a combination of two or more, of the following:

- conception and design of the project or output
- acquisition of research data where the acquisition has required significant intellectual judgement, planning, design, or input
- contribution of knowledge, where justified, including Indigenous knowledge
- analysis or interpretation of research data
- drafting significant parts of the research output or critically revising it so as to contribute to its interpretation.

According to this definition, the authors of the paper titled:
The Effects of Intermittent Fasting Combined with Resistance Training on Lean Body Mass: A Systematic Review of Human Studies
Submitted/resubmitted to: Nutrients on 10th July 2020 are the undersigned.

The order in which the authors' names appear in the submitted paper is acceptable to all authors. All authors agree that they have met the minimum requirements listed above and have approved the submitted version of the paper. All authors agree that they are responsible for the content of the paper.

Name	Accepted/Declined authorship	Signature (The Swinburne corresponding author on the paper should be marked in **bold**)	Description of contribution appended
Stephen Keenan	Accepted	[redacted]	Conceptualisation, original draft preparation, re-writing, review and editing

Regina Belski	Accepted	[redacted]	Conceptualisation, review and editing, supervision.
Matthew Cooke	Accepted	[redacted]	Re-writing, review and editing, supervision.

More rows may be added as necessary

I Stephen Keenan (executive or corresponding author) declare that all contributing parties, facilities and materials to the research have been properly acknowledged and where individuals are named, their consent has been obtained.

If for any reason, one or more of the co-authors is unavailable or otherwise unable to sign above, an email from them acknowledging that they are in agreement with the statements must be appended.

Appendix 2. *Nutrients* copyright statement.

Appendix 3. PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	1-2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	2
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	2-3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	2
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supp 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	2-3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	3
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	3
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	3-4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	3

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	3
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	3
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	4-15
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	15
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	4-15, table 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	15
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	15-16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097
For more information, visit: www.prisma-statement.org.

Appendix 4. Example of search strategy for MEDLINE for systematic review

1	(alternate day fast* or alternat* calori* diet* or alternate day diet* or alternate day modified fast* or intermittent fast* or intermittent energy fast* or intermittent energy restrict* or intermittent calori* restrict* or ADF or time restricted feed* or TRF or ramadan or ramadan fast*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	6660
2	(exercis* or resistance exercis* or training).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	740194
3	body composition.mp. or exp Body Composition/	57128
4	1 and 2 and 3	46

Appendix 5. Ethics approval emails for initial application and major and minor amendments.

All conditions pertaining to clearance were properly met, and annual reports have been submitted. Ethics approval has been extended until December, 2020 for all data-analysis and write-up, and thus the final report is due in December 2020. Furthermore, when the extension was granted, an additional investigator was added to facilitate data-analysis. All further ethics approvals are below.

Appendix 5.1. Approval for initial application

Dear Regina,

Project 2018/322 – Comparing the effects of 12 weeks resistance exercise combined with either intermittent fasting (5:2) using high protein supplements or continuous energy restriction on body composition, strength, aerobic capacity, adherence and metabolic health in moderately overweight individuals

A/Prof. Regina Belski, Mr Stephen Keenan (Student), Dr Matthew Cooke – FHAD; Dr Adrienne Forsyth (La Trobe University)

Approved duration: 28-11-2018 to 28-11-2019 [adjusted]

I refer to the ethical review of the above project protocol by Swinburne's Human Research Ethics Committee (SUHREC). Your response to the review, as emailed by Stephen Keenan on 28 November 2018, accords with the Committee review.

I am pleased to advise that, as submitted to date, the project may proceed in line with standard on-going ethics clearance conditions outlined below.

The approved duration is **28 November 2018** to **28 November 2019** unless an extension request is subsequently approved.

All human research activity undertaken under Swinburne auspices must conform to Swinburne and external regulatory standards, including the *National Statement on Ethical Conduct in Human Research (2007 – updated 2018)* and with respect to secure data use, retention and disposal.

The named Swinburne Chief Investigator/Supervisor remains responsible for any personnel appointed to or associated with the project being made aware of ethics clearance conditions, including research and consent procedures or instruments approved. Any change in chief investigator/supervisor, and addition or removal of other personnel/students from the project, requires timely notification and SUHREC endorsement.

The above project has been approved as submitted for ethical review by or on behalf of SUHREC. Amendments to approved procedures or instruments ordinarily require prior ethical appraisal/clearance. SUHREC must be notified immediately or as soon as possible thereafter of (a) any serious or unexpected adverse effects on participants and any redress measures; (b) proposed changes in protocols; and (c) unforeseen events which might affect continued ethical acceptability of the project.

At a minimum, an annual report on the progress of the project is required as well as at the conclusion (or abandonment) of the project. Information on project monitoring and variations/additions, self-audits and progress reports can be found on the Research Ethics Internet pages.

A duly authorised external or internal audit of the project may be undertaken at any time.

Please contact the Research Ethics Office if you have any queries about on-going ethics clearance, citing the Swinburne project number. A copy of this email should be retained as part of project record-keeping.

Best wishes for the project.

Yours sincerely

Astrid Nordmann

Secretary, SUHREC

Dr Astrid Nordmann | Research Ethics Coordinator

Swinburne Research| Swinburne University of Technology

Ph +61 3 9214 3845| anordmann@swin.edu.au

Level 1, Swinburne Place South

24 Wakefield St, Hawthorn VIC 3122, Australia

www.swinburne.edu.au

Appendix 5.2. Approval for minor amendments

Dear Regina,

Project 2018/322 – Comparing the effects of 12 weeks resistance exercise combined with either intermittent fasting (5:2) using high protein supplements or continuous energy restriction on body composition, strength, aerobic capacity, adherence and metabolic health in moderately overweight individuals

A/Prof. Regina Belski, Mr Stephen Keenan (Student), Dr Matthew Cooke – FHAD; Dr Adrienne Forsyth (La Trobe University)

Approved duration: 28-11-2018 to 28-11-2019 [adjusted]

Modified: February 2019

I refer to your request to modify the approved protocol for the above project as emailed by Stephen Keenan on 1 February 2019. The request (concerning small changes to the exercise plan and testing procedure, adjustment to inclusion criteria relating to the BMI of participants, addition of the Sunshine Hospital location for the DEXA scan, and corresponding revisions to the consent documentation) was put to a SUHREC delegate for consideration.

I am pleased to advise that, as modified to date, the project may continue in line with standard ethics clearance conditions previously communicated and reprinted below. Please note that information on self-auditing, progress/final reporting and modifications/additions to approved protocols can now be found on the Research Ethics Internet pages.

Please contact the Research Ethics Office if you have any queries about on-going ethics clearance, citing the project number. A copy of this email should be retained as part of project record-keeping.

As before, best wishes for the project.

Yours sincerely,

Astrid Nordmann

Dr Astrid Nordmann | Research Ethics Coordinator

Swinburne Research| Swinburne University of Technology

Ph +61 3 9214 3845| anordmann@swin.edu.au

Level 1, Swinburne Place South

24 Wakefield St, Hawthorn VIC 3122, Australia

www.swinburne.edu.au

Appendix 5.3. Approval for major amendments

Dear Regina,

Project 2018/322 – Comparing the effects of 12 weeks resistance exercise combined with either intermittent fasting (5:2) using high protein supplements or continuous energy restriction on body composition, strength, aerobic capacity, adherence and metabolic health in moderately overweight individuals

A/Prof. Regina Belski, Mr Stephen Keenan (Student), Dr Matthew Cooke – FHAD; Dr Adrienne Forsyth (La Trobe University)

Approved duration: 28-11-2018 to 28-11-2019 [adjusted]

Modified: February 2019, March 2019

I refer to your request to modify the approved protocol for the above project as emailed by Stephen Keenan on 1 February 2019. The request (concerning the use of additional avenues for recruitment; collection and analysis of faecal samples at the beginning and end of the intervention period; the use of muscle ultrasound at the beginning and end of the intervention period, and adjustment to the blood collection and storage protocol) was considered at SUHREC meeting 02/2019 held on 15 February 2019. Your responses to SUHREC review, submitted on 22 February and 01 March 2019, were also considered by SUHREC delegates.

I am pleased to advise that, as modified to date, the project may continue in line with standard ethics clearance conditions previously communicated and reprinted below. Please note that information on self-auditing, progress/final reporting and modifications/additions to approved protocols can now be found on the Research Ethics Internet pages.

Please contact the Research Ethics Office if you have any queries about on-going ethics clearance, citing the project number. A copy of this email should be retained as part of project record-keeping.

As before, best wishes for the project.

Yours sincerely,

Astrid Nordmann

Dr Astrid Nordmann | Research Ethics Coordinator

Swinburne Research| Swinburne University of Technology

Ph +61 3 9214 3845| anordmann@swin.edu.au

Level 1, Swinburne Place South

24 Wakefield St, Hawthorn VIC 3122, Australia

www.swinburne.edu.au

Appendix 5.4. Approval for ethics extension.

30/07/2020

Ref: 20200920-4827 : Comparing the effects of 12 weeks resistance exercise combined with either intermittent fasting using high protein supplements or continuous energy restriction on body composition and other parameters

Approved Duration: 28/11/2018 to 31/12/2020

Chief Investigator: Regina Belski

I refer to your request to modify the approved protocol for the above project. The request was put to a SUHREC/SHESC delegate for consideration.

I am pleased to advise that, as modified to date, the project may continue in line with standard ethics clearance conditions previously communicated and reprinted below. Please note that information on self-auditing, progress/final reporting and modifications/additions to approved protocols can now be found on the Research Ethics Internet pages.

Please contact the Research Ethics Office if you have any queries about on-going ethics clearance, citing the project number. A copy of this correspondence should be retained as part of project record-keeping and forwarded to relevant members of the project team.

This modification was approved during COVID-19 restrictions. The conduct of the research during this period should reflect any changes in relation to university and government COVID-19 mandates in the relevant jurisdictions. To ensure you have accommodated these mandates please refer to the Swinburne Ethics COVID-19 website here.

As before, best wishes for the project.

Yours sincerely,

Dr Astrid Nordmann

Research Ethics Office

Swinburne University of Technology

P: +61 3 9214 3845 | E: resethics@swin.edu.au

Appendix 6. Example meal plan for a participant in the IFT group

Dietary Plan – Intermittent Fasting

Goals and instructions

1. Choose 1 breakfast option, 1 lunch option, 1 dinner option and 1 snack option from the suggested meals below
2. You can substitute anything in and out of these meal plans as long as you hit the energy and protein requirements listed for that particular meal or snack
3. Your substituted meals must also adhere to the golden rules below
4. You can use as many herbs and spices as you like on any meal as these do not add energy
5. Try to avoid alcohol for the period of the study
6. You can consume non-caloric beverages such as diet soft drink or coffee/tea without added sugar or milk – if you want to add sugar or milk you need to check how much energy is in that and deduct that from somewhere else in the day
7. Stop taking any dietary supplements for the duration of the study
8. This meal plan is for your **NON FASTING** days – for fasting days follow the rules listed later

Golden rules:

1. You **MUST** include vegetables at both lunch and dinner
2. You **MUST** try **NOT TO** go over your energy target for the day, but do try to reach it or get close
3. You **MUST** try to hit your protein target

Energy Target	Protein Target
10,500kJ / day	120g / day

Meal Plan

Breakfast – Choose 1

All breakfast options are approximately 3000kJ and contain 30-35g of protein

Toast with peanut butter and Yoghurt with cereal	*Toast with eggs and vegetables, with fruit*	*Cereal with milk and toast with peanut butter*
• 2 slices of white or multigrain bread* • Thick spread of peanut butter on each slice • 1 x 170g Chobani flavoured yoghurt tub • 50 grams (approx. 1.5 small fist, or 3 cupped handfuls) of dry cereal on yoghurt	• 2 slices of white or multigrain bread* • 2 large eggs • Non-starchy vegetable of your choice (eg spinach, mushroom, tomato) • 1 teaspoon of oil (about the size of the tip of your thumb) for cooking • 1 medium piece of fruit • 1 cup of milk (1 fist)	• 50g (3 cupped handfuls or 1.5 fists) of Sultana Bran or other bran flake cereal OR 5 Weetbix • 350ml (~1.5 cups, or 1.5 fists) of milk • 1 slice of white or multigrain bread • 1 spread of peanut butter

* Standard loaves such as Woolworths, Tip Top, Wonderwhite – note other brands such as Abbott's Bakery or Helga's use larger slices and therefore have more energy, so you will need to adjust your serve size

Lunch – Choose 1

All lunch options are approximately 3500kJ and contain 50g of protein

Ham or tuna sandwiches	*Chicken wraps*	*Chicken and Rice*
• 4 slices of white or multigrain bread* • 10 thin shaved slices of ham (120g) OR 1 x 95g can of tuna in springwater • 2 tasty cheese slices • 1 handful of salad leaves on each sandwich • 1 handful (approximately 20) almonds	• 2 white or wholegrain wraps (eg Mission brand) • ½ a medium chicken breast** (120g or ~1 and ¼ palm sized pieces of chicken – if roast chicken remove the skin) • 2 tasty cheese slices • 1 large handful of salad leaves on each wrap • 1 teaspoon of oil (about the size of the tip of your thumb) for cooking	• 140g chicken breast** (approximately 1.4 palms) • 1-2 fists of non-starchy vegetables (for example cauliflower, broccoli, tomato, cucumber, onion) • 200g **cooked** white rice (about two fists) • Campbell's real stock chicken salt reduced 200mL • 35g Honey • 35g Mustard • 2 tsp of oil for cooking

* Standard loaves such as Woolworths, Tip Top, Wonderwhite – note other brands such as Abbott's Bakery or Helga's use larger slices and therefore have more energy.

** Chicken is breast with no skin and is raw weight. 100g of chicken or red meat is equivalent to about the size of your palm in area and in thickness.

Dinner – Choose 1

All dinner options are approximately 3500kJ and contain 50g of protein

Chicken or beef curry	***Fried Salmon***	***Chicken or Beef Burger***
• 130g beef, trimmed of fat (1.3 palms) or 150g (1.5 palms) chicken breast** • 1 fist of starchy vegetables (for example 1 potato OR 1 carrot, OR ½ potato and ½ carrot) • 1-2 fists of non-starchy vegetables (for example broccoli, cauliflower, tomato, cucumber, onion) • ¼ jar of simmer sauce (eg Patak's or Passage to India – if home-made try to match energy content to this type of sauce) • 160g of cooked rice (around 1 cup or a fist) • 1 tablespoon of oil for cooking (about the full size of your thumb)	• 160g of Salmon (about a 1.5 palm sized piece) • 1 tablespoon of olive oil for cooking (about the size of your thumb) • 2 fists of starchy vegetables (for example 2 medium potatoes or 2 carrots) OR 1 cob of corn (if mashing potatoes, use a splash of milk instead of butter or cream) • 2 fists of non-starchy vegetables (for example cauliflower, broccoli, tomato, cucumber, onion)	• 1 Mighty Soft or equivalent hamburger bun • 130g lean beef mince or 150g chicken breast** (1.5 palms) • 1 tasty cheese slice • 1 large handful of salad leaves • 2 slices of tomato • 2 slices of beetroot • 1 teaspoon of mayonnaise (about the size of the tip of your thumb) • 1 teaspoon of oil for cooking • 10-12 beer battered oven chips

** Chicken is breast with no skin and is raw weight. 100g of chicken or red meat is equivalent to about the size of your palm in area and in thickness.

Snacks – Choose 1 per day

All snacks contain around 400-600kJ

Choose most • 15 raw almonds • 1 medium apple, banana, orange or pear • 2 small apricots, kiwi fruits or plums • 1 slice of light and tasty cheese with 2 vitaweat style multigrain crackers or with 4 small dry cracker • 1 small (100-150g) tub of low fat flavoured yoghurt	• 1 medium carrot (cut into sticks) with 4 teaspoons of hummus or other dip (1 teaspoon is about the size of the last segment of your thumb) • 1 small take away coffee with full cream milk (you can have sweetener, or up to 1 sugar) • 1 medium take away coffee with skim milk (you can have sweetener, or up to 1 sugar)
Choose occasionally • 125ml (½ cup) fruit juice • 4 squares of dark or milk chocolate (25g)	• 1 small handful of lollies (25g) • 1 fun size chocolate

Fasting days – Tuesday/Thursday

Instructions

1. On your fasting day you need to consume all of your food between 12pm and 6pm – you can spread it out however you like over these hours
2. You must **NOT** fast on days when you have your exercise classes or your home work out
3. You must **NOT** fast on consecutive days
4. You are allowed to drink non-caloric beverages such as water, diet soft drink and coffee/tea with no milk or sugar added.

Consume the following between 12pm (Midday) and 6pm:

Formulite protein shakes x 2* *Follow the instructions on the pack to prepare this. Each shake should consist of 2 scoops of protein powder mixed with 200-300mL of water
Formulite soup x 1* *Follow the instructions on the pack to prepare this. Empty one sachet into a bowl or mug then add 250mL of hot water then consume
Bird's eye frozen vegetables 1 x 150g frozen vegetable bag or 1 cup (1 fist) of raw/steamed vegetables.

Appendix 7. Supervised exercise program.

Warmup

5-10 minute warm up – stretching, jogging on the spot.

Workout

Supersets (exercise 1 superset with exercise 2 in each exercise pair). Continue until they can complete 3 sets of 12-15 reps of each exercise (aim to hit minimum of 12 repetitions), then progress. 2 minutes rest between sets.

Superset 1

Exercise 1

Push ups

Progressions:

1. Wall push ups (Keep distance from wall consistent/standard with all participants)
2. Knee push ups
3. Standard push ups
4. Depth push up (performed on weights)
5. Elevated push up (legs elevated)
6. Weighted (plate on back)

Exercise 2

Squats

Progressions:

1. Bodyweight parallel squat
2. Bodyweight full squat
3. Goblet squat (with dumbbell) parallel
4. Single leg movement (e.g Bulgarian) with or without dumbbells (for example, if they can do single leg and achieve 15 repetitions easily, then add dumbbells so that they can just reach 12 repetitions)

Superset 2

Exercise 1

Bent over rows

Progressions:

1. Resistance bands

2. Dumbbells/barbells – progression to heavier dumbbells/barbells

Exercise 2

Lunges

Progressions:

1. Static lunge (with or without weight)
2. Forward lunge
3. Forward lunge with weight (2 dumbbells or weight plate and progress heavier)
4. Jump split squat

Superset 3

Exercise 1

Bicep curls

Progressions:

1. Resistance band curls
2. Dumbbell curls – progress to heavier dumbbells

Exercise 2

Dips

Progressions:

1. Bent knee chair/bench dips
2. Straight leg chair/bench dips
3. One leg raised chair/bench dips
4. Both legs elevated chair/bench tips
5. Dumbbell on lap elevated leg chair/bench dips – progress to heavier dumbbells

Home workout

Progressions made by increasing time on each exercise by 5 seconds each week. 2 minutes rest between each superset, 3 sets of each superset.

Superset 1

30 seconds plank

30 seconds mountain climbers

Superset 2

30 seconds crunches/sit ups

30 seconds burpees

Superset 3

30 seconds side toe touches

30 seconds hip bridge

Cool down

5 minutes stretching

Appendix 8. Daily surveys questions for hunger, mood, energy levels and compliance for IFT group

*Note: this questionnaire was adapted into an online version to facilitate easier completion.

Date:

*Indicate below (**circle number**)* that corresponds to how you feel currently:

How hungry do you feel?

I am not hungry at all	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**I have never been more hungry**

How satisfied do you feel?

I am completely empty	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**I cannot eat another bite**

How full do you feel?

I am completely empty	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Totally full**

How much do you think you can eat?

Nothing at all	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**A lot**

Would you like something sweet?

Yes, very much	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**No, not at all**

Would you like something salty?

Yes, very much	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**No, not at all**

Would you like something savoury?

Yes, very much	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**No, not at all**

Would you like something fatty?

Yes, very much	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**No, not at all**

Daily Mood Scale

*Indicate below (**circle number**)* that corresponds to how you feel currently:

How alert do you feel?

Very little 0 1 2 3 4 5 6 7 8 9 10 Very much

How sad do you feel?

Very little 0 1 2 3 4 5 6 7 8 9 10 Very much

How tense do you feel?

Very little 0 1 2 3 4 5 6 7 8 9 10 Very much

How much of an effort is it to do anything?

Very little 0 1 2 3 4 5 6 7 8 9 10 Very much

How happy do you feel?

Very little 0 1 2 3 4 5 6 7 8 9 10 Very much

How weary do you feel?

Very little 0 1 2 3 4 5 6 7 8 9 10 Very much

How calm do you feel?

Very little 0 1 2 3 4 5 6 7 8 9 10 Very much

How sleepy do you feel?

Very little 0 1 2 3 4 5 6 7 8 9 10 Very much

How compliant do you feel you were with your diet today?

Not compliant at all 0 1 2 3 4 5 6 7 8 9 10 Fully compliant

The questions in the below section appeared only for those in the IFT group

Did you fast today? (Y / N) (*this question*

If yes:

Did you consume:

Protein shake 1: (Y / N)

Protein shake 2: (Y / N)

Soup: (Y / N)

Vegetables: (Y / N)

Did you consume these between 12pm and 6pm? (Y / N)

If any, what excess foods/drinks did you consume and what time did you consume them? (If you feel you were fully compliant, you can skip this question)

Appendix 9. Post-intervention questionnaire

Date:

*Indicate below (**circle number**)* that corresponds to how you feel currently:

	How easy was it to adhere to your diet?											
Not easy at all	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Extremely easy**
	How energetic did you feel throughout the intervention?											
I had no energy at all	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**I always had a lot of energy**
	How energetic did you feel during your workouts throughout the intervention?											
I had no energy at all	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**I always had a lot of energy**

Will you continue with your diet after the intervention period has finished? (Y / N)

What did you find to be the most difficult part of your diet, and in what situations was it most difficult to continue to adhere to your diet?

____

____

____

What was the best part about the diet, and what made it easier to adhere to your diet?

____

____

____

What was the most difficult part of the exercise intervention?

____

____

____

What was the best part about the exercise intervention?

Any other feedback?

Would you have preferred to have been in the other diet group? (Y / N)

Appendix 10. Shortened version of body composition paper submitted to the Journal of Nutrition.

Intermittent fasting and continuous energy restriction result in similar changes in body composition and muscle strength when combined with a 12 week resistance training program

Authors

Stephen J. Keenan[1], Matthew B. Cooke[1,2], Ebrahim Bani Hassan[2,3], Won Sun Chen[1], Josef Sullivan[1], Sam X. Wu[1], Doa El-Ansary[1,4], Mahdi Imani[2,3], Regina Belski[1]

1. Faculty of Health, Arts and Design, School of Health Sciences, Swinburne University of Technology, Melbourne, Australia.
2. Australian Institute for Musculoskeletal Science (AIMSS), The University of Melbourne, VIC, Australia.
3. Department of Medicine, Western Health, Melbourne Medical School, The University of Melbourne, VIC, Australia.
4. Department of Surgery, Melbourne Medical School, The University of Melbourne, VIC, Australia

Corresponding author: Stephen J. Keenan – skeenan@swin.edu.au

Conflict of interest: Stephen J. Keenan -no conflict of interest; Matthew B. Cooke – no conflict of interest; Ebrahim Bani Hassan – no conflict of interest; Won Sun Chen – no conflict of interest; Josef Sullivan – no conflict of interest; Sam Wu – no conflict of interest; Doa El-Ansary – no conflict of interest; Mahdi Imani – no conflict of interest; Regina Belski – no conflict of interest.

Word count: 4959

Number of figures: 2

Number of tables: 6

Supplementary data: Supplementary file 1.

Running title: Energy restriction and resistance training

List of abbreviations:

CSA, cross-sectional area; CERT, continuous energy restriction plus training group; DXA, dual x-ray absorptiometry; EI, echogenicity; IFT, intermittent fasting plus training group; LBM, lean body mass; MPB, muscle protein breakdown; MPS, muscle protein synthesis; pQCT, peripheral quantitative computed tomography; RF, rectus femoris; VI, vastus intermedius; 1RM, 1-repetition maximum; 3RM, 3-repetition maximum.

Abstract

Background: Energy restricted diets commonly lead to loss of lean body mass (LBM), however resistance training and increased protein intake can help attenuate these losses.

Objective: The objective of this study was to compare the effects of 12 weeks of resistance training combined with either 5:2 intermittent fasting or continuous energy restriction on body composition, muscle size and quality, and upper and lower body strength.

Methods: Untrained individuals were randomly assigned to resistance training plus either continuous energy restriction [20% daily energy restriction (CERT)] or 5:2 intermittent fasting [~70% energy restriction 2 days/week, euenergetic consumption 5 days/week (IFT)] groups, with both groups prescribed an average of ≥ 1.4 grams of protein per kilogram of body weight per day. Participants completed 2 supervised resistance and 1 unsupervised aerobic/resistance training combination sessions per week for 12 weeks. Changes in LBM were assessed as a primary outcome. Other body composition changes, thigh muscle size and quality, strength and dietary intake were assessed as secondary outcomes.

Results: Thirty-four participants completed the study (CERT; n = 17, IFT; n = 17). At the end of the 12 weeks, LBM was significantly increased (+3.7%, p<0.0001) and both body weight (-4.6%, p = <0.0001) and fat (-24.1%, p<0.0001) were significantly reduced with no difference between groups, though results differed by sex. Both groups showed improvements in thigh muscle size and quality, and reduced intramuscular and subcutaneous fat assessed by ultrasonography and peripheral quantitative computed tomography (pQCT), respectively. However, only the CERT group demonstrated a significant increase in muscle surface area assessed by pQCT. Similar gains in upper and lower body strength and muscular endurance were observed in both groups.

Conclusion: When combined with resistance training and moderate protein intake, continuous energy restriction and 5:2 intermittent fasting resulted in similar improvements in body composition, muscle quality, and strength.

Key words: Intermittent fasting; continuous energy restriction; resistance training; body composition; lean body mass; intramuscular fat; weight loss.

Introduction

Energy restricted diets are increasingly popular amongst individuals for a variety of reasons ranging from improving body composition to general health and wellbeing. Regardless of the reason, these diets commonly lead to weight loss in the form of both fat and lean body mass (LBM) (1). While fat loss is usually desirable, reductions in skeletal muscle mass (a major component of LBM) may lead to a number of deleterious short- and long-term consequences, such as hyperphagia and reduced basal metabolic rate, which may compromise long term weight loss success (2); increased risk of strength loss and disability, especially in older adults (3,4); and potential metabolic issues incumbent with low muscle mass (5).

The mechanisms behind weight-loss induced reductions in LBM are not fully understood, however, the impact of energy restriction on protein turnover and net muscle protein balance may be a contributing factor (6). Skeletal muscle mass is determined by the balance of muscle protein synthesis (MPS) and muscle protein breakdown (MPB), which remains equal during energy balance (7). Conversely, during short-term, continuous energy restriction, both post-prandial and post-absorptive MPS are reduced (8), which may lead to an overall negative protein balance, higher protein catabolism to supply amino acids and reductions in muscle mass (6). However, whether this also occurs over extended periods of energy restriction is unclear (9). Notwithstanding, higher protein intakes, and/or performing resistance training have been shown to partially or completely attenuate these reductions in MPS (6,10). Moreover, these strategies, as well as others including slower rates of weight loss, have been utilised to successfully mitigate LBM loss during longer periods of energy restriction (1,11).

Recently, the pattern of energy restriction has emerged as another potential method for preserving LBM. Compared to traditional energy restricted diets that are characterised by moderate daily energy restriction (i.e. continuous energy restriction), alternative patterns such as intermittent fasting, which intersperse periods of severe energy restriction with regular dietary or ad libitum consumption have gained recent attention. It has been hypothesised that intermittent fasting may be protective of LBM loss compared to continuous energy restriction (12), though some argue that severe energy restriction, even if acute, could lead to greater reductions in LBM (13). Notwithstanding, it is clear that the majority of energy restricted diets, regardless of the pattern, can still lead to LBM loss (14), and thus should be combined with other strategies that are known to preserve and/or promote muscle growth.

While there are many variations of intermittent fasting, the 5:2 style diet, which generally involves 2 days per week of severe (consumption of ~1,600-3,000 kJ/day) or complete energy restriction, paired with 5 days of ad libitum or euenergetic consumption (15–26), has received limited attention in the context of LBM preservation. To date, no studies have compared the effects of a 5:2 style diet to continuous energy restriction on body composition and muscle strength adaptations when both are combined with a resistance training program. Furthermore, only a handful of intermittent fasting studies have utilised more sensitive assessments of muscle hypertrophy (i.e. ultrasonography) to simultaneously assess changes in muscle growth (27–30). Thus, the purpose of this study was to investigate and compare the effects of 12 weeks of resistance training combined with either a 5:2 fasting or continuous energy restriction style diet on body composition, especially LBM, indicators of muscle hypertrophy and quality, and upper and lower body strength.

Methods

Participants

Participants were recruited through advertising on social media channels targeting current and past university students in Victoria, Australia. A total of 194 individuals responded to the advertisement and underwent initial screening. Only 44 were deemed eligible and were recruited for the study. Participants were eligible for inclusion if they: (i) were aged between 18-45 years; (ii) had a body mass index (BMI) of 22.0-35.0kg/m^2; (iii) had a body fat percentage >18% for males or >25% for females as measured via dual x-ray absorptiometry (DXA); (iv) had not followed a structured resistance training program in the previous 6 months and; (v) had been weight stable for 3 months prior to the study (<5% weight loss or weight gain).

Randomisation and Study Overview

Participants who were eligible for the study were stratified by age, sex and BMI before being randomised by coin toss into either the intermittent fasting plus training (IFT) or continuous energy restriction plus training (CERT) groups for 12 weeks. Participants in the IFT group undertook a 5:2 style-fasting protocol, while those in the CERT group undertook a continuous energy restriction style feeding pattern. Both groups completed supervised resistance training twice per week, and resistance/aerobic combination training once per week. The intervention took place from February to November, 2019, spread across 6 groups, starting 2-4 weeks apart. A flow chart showing participant movement through the study can be seen in **Figure 1**. This study was approved by the Swinburne University of Technology Human Research Ethics Committee (project #2018/322).

Diet Protocol

Basal energy requirements for all participants were calculated using the Mifflin St. Jeor equation (31), with total energy requirements calculated by applying an activity factor of 1.4 representing a recreational level of activity (based on prescribed exercise). At the beginning of the intervention period, all participants were provided with example meal plans that would result in average consumption of approximately 80% of estimated energy requirements and 1.4 grams of protein per kilogram of body weight per day (g/kg/day). Meal plans were customised based on food preferences for each individual and provided by a dietitian along with brief education on the Australian healthy eating guidelines (32). As participants in the IFT group had limited energy available on fasting days to reach recommended protein intakes, they were provided with protein shakes and high protein soups in order to get as close to daily protein intake recommendations as possible. Participants were also instructed on how to use the Easy Diet Diary (Xyris Software, Australia, 2019) smart phone application to record their food, and substitute other foods of their choosing into the meal plans, while maintaining the same energy and protein intake.

Intermittent Fasting Diet Protocol

All participants in the IFT group were instructed to consume 100% of their energy requirements for 5 days per week (non-fasting days). On two non-consecutive and non-training days, participants consumed approximately 30% of their estimated energy requirements (~2,100 kJ for females and ~2,500 kJ for males) (fasting days), consistent with previous research (33,34). On fasting days, participants were prescribed a diet consisting of whey-based protein shakes (Formulite), high-protein soups and steamed/raw vegetables. The macronutrient composition of these supplements and the recommended intake on fasting days can be seen in **Table 1**. On fasting days, participants were asked to consume all energy during a 6 hour window between 12.00 pm and 6.00 pm and were allowed ad libitum consumption of non-energy providing beverages. In order to match protein intakes across dietary groups as closely as possible, those in the IFT group were instructed to consume approximately 1.5 g/kg/day of protein on non-fasting days, as their fasting day diets only provided ~1.1-1.2 g/kg/day.

Continuous Energy Restriction

Those randomised to the CERT group were instructed to consume ~80% of their total energy requirements daily for the duration of the 12 week intervention. Furthermore, participants were also prescribed consumption of 1.4 g/kg/day of protein. Participants in this group also received customised meal plans and the same education as those in the IFT group.

Exercise Protocol

All participants were required to undertake 3 training sessions each week: 2 resistance training sessions and 1 body weight aerobic/resistance training combination session. The two resistance training sessions were conducted at Swinburne University's Hawthorn campus, and were supervised by an accredited strength and conditioning coach (who was also the study dietitian). The 2 supervised sessions consisted of variations of the following exercises: push-ups, squats, rows, lunges, bicep curls and dips (Supplemental Figure 1). Participants completed these exercises in a superset style workout, aiming to complete 12-15 repetitions of each exercise. Once participants were able to complete 3 sets of 15 repetitions in any individual exercise, the weight was increased or exercise variation made more difficult, adhering to the principles of progressive overload. The one body weight aerobic/resistance training combination session per week was completed by participants at home using body weight exercises consisting of: planks, mountain climbers, crunches, burpees, lying side toe-touches and hip bridges. These exercises were also completed using a superset format with 2 minute breaks, however were timed instead of counting repetitions. When participants reached their time goal with good form (self-assessed), they were instructed to increase this by 5 seconds.

Assessments

Body weight and body composition analysis

Weekly weight measurements were taken in light clothing using bioelectrical impedance scales [Multifrequency segmental body composition analyser; MC-780 Tanita Corporation (Tokyo, Japan)]. Lean body mass, fat mass and body fat percentage were assessed utilising dual x-ray absorptiometry [DXA; Hologic Horizon (Bedford MA)] at baseline and after the intervention period, as previously detailed (35). The DXA was calibrated for bone mineral density, muscle and fat masses on the morning of each assessment in accordance with manufacturer guidelines using spine and whole-body phantoms respectively.

Ultrasound

Muscle thickness, cross-sectional area (CSA) and muscle quality [echogenicity (EI)], were measured using ultrasound (SonoSite M-Turbo, SonoSite Australasia Pty Ltd, New South Wales, Australia) with a linear array transducer (5-2 MHz) for the rectus femoris (RF) and vastus intermedius (VI) of the non-dominant leg at baseline and post intervention (Supplemental Figure 2). All images were acquired and analysed by the same technician. A fidelity check of the image acquisition and analysis was conducted by health professional with over 10 years-experience in ultrasound imaging and analysis. Varying depths were required to obtain full visualisation of the RF in some instances, however gain was kept consistent across measurements. Measurements were acquired with participants in a supine position, with their knee in passive extension. Ultrasound gel was applied to the transducer, which was placed perpendicular to the long axis of the anterior thigh, at a distance of two thirds from the anterior, superior iliac spine to the superior patellar border, consistent with previous studies (36). Muscle thickness and CSA were measured in real-time with the on-board functions of the M-turbo, utilising the straight line and tracing functions respectively. Images were also saved onto the on-board hard-drive before being transferred onto a personal computer for EI analysis using ImageJ software (NIH, Bethesda, MD) (37). EI for RF and VI were measured utilising a standard square of 100 x 100 pixels, or where the predefined square did not fit within the cross section of the muscle, the largest square that fit within the anatomic boundaries of the muscle was utilised, a method which has shown good inter-observer reliability regardless of level of expertise (38).

Peripheral Quantitative Computed Tomography (pQCT)

pQCT was utilised to measure the surface area of muscle, intramuscular fat and subcutaneous fat at baseline and post intervention (Supplemental Figure 3). A single 2.5 mm transverse pQCT; (Stratec XCT3000, Stratec Medizintechnik GmbH, Pforzheim, Germany) scan with a voxel size of 0.4 mm was obtained at mid-thigh region of the non-dominant leg. The mid-thigh was defined as midway between the tip of the greater trochanter and medial edge of the tibial plateau, located by deep palpation. The images were exported and further analyzed by Slice-O-Matic™ (Tomovision, Montreal, CA) to determine the muscle, intramuscular fat and subcutaneous fat volumes as previously described (39–41). After visual checks; where due to beam hardening artefacts the tissue was not segmented ("tagged") optimally, the assignment of individual voxels or small voxel islands were changed into the correct tissue manually, at the discretion of the operator.

All imaging and image analyses were carried out by a single experienced image analysis specialist (EB) or under his direct supervision.

Strength testing

Strength testing was undertaken prior to the diet intervention and at the end of week 12. A 3 repetition maximum (3RM) test and strength endurance test were performed for both bench press and leg press. After a brief 5 minute warm up, participants were instructed on correct lifting and breathing techniques before practicing these using submaximal loads for 10-15 repetitions. Weight was gradually added and repetitions reduced to serve as a functional warm up. Participants then completed a set of 3 repetitions at a self-selected weight close to their perceived capacity, followed by a 3 minute rest, with weight being continually added to each subsequent attempt. 3RM was recorded as the last successful attempt before form breakdown, or failure to complete the lift without assistance. The 3RM of each participant was determined within 5 attempts. After the 3RM test, participants were allowed a 5 minute rest before undergoing a strength endurance test. These were tested in order such that 3RM bench press was followed by the bench press endurance test, and 3RM leg press was followed by the leg press endurance test. Participants were required to complete as many repetitions as possible of each exercise utilising 70% of their estimated 1 repetition maximum (1RM), calculated from the attained 3RM utilising the Brzycki formula (42) (weight lifted/[1.0278 – (0.0278 x repetitions performed)]). Failure was determined as the first repetition where the participant required assistance. Repetitions where form was considered inadequate were not counted; however, participants were not stopped from completing subsequent repetitions if this occurred. Volume was calculated as the number of repetitions completed at 70% of 1RM multiplied by weight lifted.

Dietary Intake

Participants were required to keep a 3 day food diary at baseline and in week 1, 6 and 12 using the Easy Diet Diary (Xyris Software, Australia, 2019) phone application. Participants recorded all food and drink intake on non-consecutive days that included 2 weekdays and 1 weekend day. Food records were kept on non-fasting days for those in the IFT group. On fasting days, participants were asked to note down any extra food or drink consumed, and whether they had consumed their recommended supplements. Intake on these days was estimated from these records.

Statistical Analysis

Results are presented as mean (± SD). Normality was assessed using the Shapiro-Wilk test and visual inspection of Q-Q plots. Assumptions of normality were violated for intramuscular fat only. Intramuscular fat was log10 transformed, resulting in normality. Linear mixed models were used to analyse variables for main effects of time and group, and time x group, time x sex and time x group x sex interactions. Differences between groups at baseline were analysed using independent t-tests. Bivariate correlations using Pearson's correlation co-efficient were calculated to assess relationships between variables and changes in LBM. All analyses were performed using SPSS version 25 (IBM Corp, Armonk, NY, 2017). A $p<0.05$ was considered significant for all tests.

Results

Participant characteristics

There were a total of 17 completers in each group, with a nearly even split of males and females (IFT = 9 males and 8 females, CERT = 8 males and 9 females). Overall, 10 participants (n = 8 female and n = 2 male) failed to complete the interventions (CERT = 5, IFT = 5). Of these 10, 3 were unable to commit to the exercise sessions, 2 were unable to commit to the dietary protocol (IFT = 1, CERT = 1), and the remaining 5 dropped out due to unrelated medical issues or relocation. Baseline characteristics for participants are presented in **Table 2**. No significant differences were found between groups as a whole, or when split by sex (not reported).

Body weight and body composition analysis

Body weight and body composition measured before and after the intervention are presented in **Table 3**. There was a main effect for time for weight [$F(1, 30) = 72.25$, $p<0.001$], BMI [$F(1, 30) = 65.45$, $p<0.001$], body fat mass [$F(1, 30) = 150.00$, $p<0.001$], body fat percentage [$F(1, 30) = 215.69$, $p<0.001$] and LBM [$F(1, 30) = 42.47$, $p<0.001$], with significant reductions in weight, BMI, body fat mass and body fat percentage observed in both dietary groups, whereas LBM was significantly increased in both groups. A significant time x sex interaction was evident for weight [$F(1, 30) = 13.12$, $p=0.001$], BMI [$F(1, 30) = 8.47$, $p=0.007$] and LBM [$F(1, 30) = 10.41$, $p=0.003$], with larger reductions in weight and BMI observed in males, but greater increases in LBM observed in females.

Mid-thigh muscle surface area and intramuscular and subcutaneous fat analysis

pQCT

Muscle surface area and intramuscular and subcutaneous fat measured before and after the intervention via pQCT are reported in **Table 3**. A main effect for time was found for subcutaneous fat [$F(1, 27) = 21.26$, $p<0.001$] and log10 intramuscular fat [$F(1, 27) = 7.13$, $p=0.01$] with significant reductions occurring in both groups over time. There was a time x group effect for muscle surface area, with those in the CERT group experiencing a mean increase in muscle surface area compared to the IFT group [$F(1, 27) = 5.65$, $p=0.03$].

Ultrasound

RF thickness, CSA and EI, and VI thickness and EI measured before and after the intervention via ultrasound are presented in **Table 3**. A main effect for time for RF thickness [$F(1, 23) = 36.90$, $p<0.001$], RF CSA [$F(1, 23) = 44.35$, $p<0.001$] and RF EI [$F(1, 23) = 17.18$, $p<0.001$] was noted, with RF thickness and CSA significantly increased in both groups over time, whereas RF EI significantly decreased in both groups over time. There were no other significant interactions or main effects identified for RF measurements and/or all VI assessments.

Dietary intake analysis

Participant dietary intake data measured before and during the intervention are summarised in **Table 4**. There was a main effect for time for overall average absolute daily energy intake in kJ [$F(1, 30) = 9.61$, $p=0.006$], relative energy intake in kJ/kg [$F(1, 30) = 6.34$, $p=0.008$], relative protein intake in g/kg [$F(1, 30) = 22.72$, $p<0.001$], relative carbohydrate intake in g/kg [$F(1, 30) = 14.34$, $p=0.001$] and relative fat in g/kg [$F(1, 30) = 16.67$, $p<0.001$], with significant reductions in energy (both absolute and relative), relative carbohydrate and fat intake identified across all groups, and a significant increase in relative protein intake. A significant time x sex interaction was found for relative energy intake (kj/kg) [$F(1, 30) = 5.64$, $p=0.03$] and relative carbohydrate intake (g/kg) [$F(1,30) = 7.56$, $p=0.01$], with females demonstrating greater reductions in energy and carbohydrate intake compared to males during the intervention period.

Fasting versus non-fasting days in IFT participants

Differences in dietary energy and protein intake on fasting and non-fasting days for the IFT group are presented in **Table 5**. Energy intake on fasting days for females was significantly lower in week 12 compared to week 1 (p=0.009). No other significant differences between time points on fasting or non-fasting days were found.

Upper body and lower body 3RM strength and endurance volume analysis

Changes in bench press and leg press 3RM, and bench press and leg press endurance test volume are reported in **Table 6**. There were main effects for time for bench press 3RM [$F(1, 30) = 75.37$, p<0.001], bench press volume [$F(1, 30) = 46.06$, p<0.001], leg press 3RM [$F(1, 30) = 84.91$, p<0.001] and leg press volume [$F(1, 30) = 39.32$, p<0.001], with increases noted in each of these variables across the intervention. No other significant interactions or main effects were identified for strength or endurance variables.

Correlations between variables and changes in LBM

Figure 2 shows the correlation between relative changes in body weight and body fat and LBM. There was a significant, moderate positive correlation between changes in LBM and weight in both groups overall (r = 0.63, p<0.001), indicating that as weight loss increased, gains in LBM reduced. When split by intervention group, this relationship was strengthened in the CERT group (r=0.86, p<0.001), while no significant relationship was seen for the IFT group (r = 0.23, p=0.38), with all but one IFT group participant increasing LBM regardless of percentage weight loss. Similarly, there was a moderate correlation between the percentage of overall fat mass lost with changes in LBM in the CERT group (r = 0.53, p=0.03), but not in the IFT group (r = -0.21, p=0.41). Change in LBM was negatively correlated with relative (kJ/kg) energy intake (r = -0.40, p=0.02) and carbohydrate (g/kg) intake (r = -0.36, *p*=0.03) and positively correlated with percent change in pQCT muscle area (r = 0.51, *p*=0.003). Relative change (percent) in bench press 3RM (r = 0.476, *p*=0.004) and leg press 3RM (r = 0.550, *p*=0.001) were also positively correlated with changes in LBM. No other measurements were found to have significant correlations with change in LBM.

Discussion

To the authors' knowledge, this is the first randomised trial to compare the effects of 5:2 intermittent fasting and continuous energy restriction on body composition and strength adaptations when concurrently undertaking resistance training. Our findings suggest that despite different patterns of energy restriction, when overall dietary energy and protein intake are similar, both diets induce comparable increases in LBM and strength, and reductions in weight and fat over a 12 week period. In contrast, assessment of the mid-thigh CSA by pQCT demonstrated greater increases in the CERT group, which was supported by similar changes in RF thickness and CSA assessed by ultrasonography; albeit not significantly. Both groups however, experienced comparable reductions in intramuscular and subcutaneous fat and improvements in muscle quality (EI). It was clear that there were sexual dimorphic differences in weight and LBM, with males demonstrating greater weight loss, but less gains in LBM, which may be a reflection of weight-loss-induced impairment of LBM accrual.

In the current study, both dietary intervention groups experienced significant reductions in body fat (mean -7.2 kg) and increases in LBM (mean +1.7 kg) suggesting weight lost was exclusively from fat. These observations were supported by localised changes in the thigh muscle with increases in RF thickness and CSA and improvements in muscle quality as indicated by decreases in RF EI, and reductions in intramuscular and subcutaneous fat measured by ultrasonography and pQCT, respectively. The changes in pQCT muscle surface area were positively correlated with changes in whole body LBM (r = 0.51, p=0.003), suggesting muscular hypertrophy may have underpinned the increases in LBM. Taken together, these findings support the notion that muscle accrual can occur together with reductions in fat mass, especially when energy deficits are combined with resistance training and protein intakes above standard daily recommendations (43–45). However, some notable differences between sexes in terms of the magnitude of change were observed. Female participants gained significantly more LBM on average (2.5 kg versus 0.9 kg, p=0.003) compared to males, but experienced less weight (-2.2 kg versus -5.5 kg, p=0.001) and fat loss (-6.4 kg versus -8.0 kg, p=0.18), though the latter was non-significant. Conversely, no significant differences between sexes were evident in variables assessed by ultrasound and pQCT. A number of sex-specific factors may explain the disparity in weight and fat reduction between males and females. Participants in the CERT group were prescribed a relative energy deficit of 20% of total estimated energy expenditure, while in the IFT group this ranged from 20-23% due to the standardised nature of fasting days. Nonetheless, this would have led to a greater absolute energy deficit for males by ~600 kJ/day given their comparatively larger lean mass. While this amount may seem small, given that the reported energy deficit for both sexes was ~10% more than prescribed (~30%), the absolute difference between sexes was most likely greater. When combined with the fact that females may require a greater absolute energy deficit per unit of weight-loss (46), are more likely to under-report energy intake (47) and may be impacted by hormones that promote weight retention (48,49), it is not surprising that males experienced greater weight and fat loss in the current study.

The differences in total amounts (and therefore overall rate) of weight loss between sexes could also explain the greater LBM gains in females compared to males. Previous research has shown that slower rates of weight loss may be beneficial for LBM preservation, though this finding is not ubiquitous in the literature (50). Congruent with this, when all weight loss and LBM changes for both sexes were pooled together in the current study, there was a moderate, positive correlation between change in weight and change in LBM ($r = 0.63$, $p<0.001$). That is, as weight loss increased, LBM gains were reduced and potentially compromised. However, when split by diet, this relationship strengthened in the CERT group ($r = 0.86$, $p<0.001$), but weakened in the IFT group to be statistically non-significant ($r = 0.23$, $p=0.38$). This trend was also seen when comparing relative overall fat loss with changes in LBM. These findings may suggest that when paired with resistance training, the 5:2 style diet could be protective/promote gains in LBM compared with continuous energy restriction when greater amounts of weight loss occur.

On the contrary, analysis of the local thigh musculature may not support this idea. Ultrasound assessment showed a greater increase in RF thickness in the CERT compared to the IFT group (CERT = + 11.2% versus IFT = +5.9%, $p=0.05$), with a similar trend for RF CSA (CERT = +19.2% versus IFT = +9.2%, $p=0.07$). Likewise, pQCT showed significantly greater increases in muscle area in the CERT group compared to IFT group (CERT = + 4.2% versus IFT = 0.0%, $p=0.03$). Disparity between groups observed at the whole LBM compared to local musculature level could be due to a number of reasons. Firstly, it is known that hypertrophy in response to resistance training does not occur uniformly throughout the body, with upper body more responsive than lower body muscle mass (51–53). It is likely that we missed changes in upper body musculature between groups by only assessing the thigh muscle utilising pQCT and ultrasound. In support of this, it was noted that when examining DXA-derived regional differences in LBM, the IFT group showed greater increases in the upper body compared to those in the CERT group, albeit marginally, while the opposite was true for the lower body (not reported). Secondly, our small, uneven sample size for the more sensitive assessments of muscle may have been insufficient to identify real differences between diet groups, especially given the known large inter-individual variability in adaptations to resistance training (54).

Despite the noted differences in body composition changes, both dietary groups and sexes demonstrated significant increases in upper and lower body muscle strength and endurance over the 12 week period. While no time x sex interaction was found for any strength/endurance variable, when expressed as a percentage change from baseline, females appeared to show greater gains which is consistent with some (55,56), but not all (51,57) previous research. This can potentially be explained by greater increases in LBM in females, and lower initial levels of strength (58) both in absolute terms and relative to LBM. Indeed, while we found a moderate positive correlation between LBM and increases in the 3RM for both bench press ($r = 0.476$, $p=0.004$) and leg press ($r = 0.550$, $p=0.001$), females also showed greater strength increases in each of these lifts expressed relative to LBM. While previous research has shown that males tend to be stronger than females per unit of LBM (59), others have suggested that when previous levels of physical activity are similar, differences in strength are almost entirely related to muscle size (60,61). Although our study specifically excluded participants who had followed a structured resistance training program in the previous 6 months, we did not investigate levels of physical activity prior to this. Thus, while speculative, it is possible that males had greater familiarity with the types of exercises performed, or were generally more physically active prior to the study, which may have resulted in a reduced relative response in strength.

There were a number of strengths of the current study. Firstly, participants were supervised for the majority of their training sessions, allowing standardisation of form and effort. Secondly, frequent access to the study dietitian may have helped improve motivation and compliance. Thirdly, we utilised non-invasive and reliable methods of assessment to measure changes in muscle size and quality alongside whole body LBM (pQCT and ultrasound), both of which have shown utility in clinical settings (62,63).

Our study was also subject to a number of limitations. Firstly, while frequent supervision and feedback for training and diet may have helped with study validity, this may not be practical for the general population. Secondly, although we collected detailed dietary data, the limitations of self-reported dietary intake are well known, and our ability to make strong inferences based on dietary data were limited. However, reported intake did reflect the aims of our study (i.e. increased intake on non-fasting days for the IFT compared to the CERT group). Thirdly, we studied recently untrained individuals only, and our results cannot be generalised to those with differing training histories. Fourthly, it is unclear how the provision of supplements to the IFT group on fasting days may have affected compliance, or whether the supplements themselves may have contributed to the results. Finally, as we did not record menstrual cycle for females, it is unclear whether this may have affected our results.

In summary, 5:2 intermittent fasting or continuous energy restriction combined with moderate protein intake and resistance training over 12 weeks led to comparable LBM gains, weight and body fat loss, and improvements in muscle strength, endurance and quality. However, there were clear sexual dimorphic differences in a number of these outcomes, and some disparities noted between measures of body composition and local muscle changes. Interestingly, there did appear to be some benefit to the 5:2 style energy restriction for LBM as total weight loss increased, however the reason for this is not immediately clear. Therefore future studies should investigate the longer-term impacts of 5:2 fasting in comparison to continuous energy restriction, but importantly, explore the relationship between transient changes in energy balance and protein turnover, weight loss and muscle mass accrual.

Acknowledgements: The authors would like to acknowledge Formulite for donating all supplement shakes and soups used in the study. This study received in-kind support of the Imaging and Image Analysis Centre at the Australian Institute for Musculoskeletal Science (AIMSS).

Author contributions:

SK, RB, MC, SW, DE designed research. SK, RB, MC, EB, MI conducted the research. WSC, JS, SK performed the statistical analyses. SK, MC, RB wrote the paper; all authors contributed to the final manuscript. SK, MC, RB had primary responsibility for the final content.

All authors have read and approved the final manuscript - pending

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Tables

Table 1. Composition of fasting day meals consumed by IFT group and overall intake on fasting days. (Original in this manuscript)

	Male	**Female**
Foods	2 x meal replacement shakes 1 x high protein soup 150g raw/steamed vegetables	1.5 x meal replacement shakes 1 x high protein soup 150g raw/steamed vegetables
Nutrients		
Energy (kJ/kcal)	2511/597	2080/495
Protein (g)	93.6	76.7
Carbohydrates (g)	30.4	26.3
Fat (g)	10.0	8.0

Table 2. Baseline participant characteristics. (Original to this manuscript)

Baseline variables	IFT Males (n = 9)	CERT Males (n = 8)	IFT Females (n = 8)	CERT Females (n = 9)	*p*-value[1]
Age (years)	25.2 (6.2)	23.1 (2.6)	24 3 (2.9)	23.2 (4 9)	0.31
Height (m)	1.81 (0.1)	1.79 (0.1)	1.62 (0.1)	1.64 (0.1)	0.82
Weight (kg)	87.3 (12.5)	88.0 (11.5)	71.9 (10.7)	72.1 (10.6)	0.92
BMI (kg/m^2)	26.6 (3.0)	27.6 (2.4)	27 5 (2.5)	26.7 (3.4)	0.93
LBM (kg)	64.4 (7.2)	64.6 (9.4)	43.0 (5.6)	43.2 (4.8)	0.82
Body Fat Percentage (%)	29.4 (6.0)	30.4 (3.7)	43.0 (3.9)	42.2 (6 3)	0.76
Bench press 3RM (kg)	58.0 (12.1)	57.0 (12.9)	26.8 (4.9)	23.9 (5.6)	0.56
Bench press volume (70% 1RM) (kg)	610.6 (197.0)	522.1 (127.6)	283.4 (104.1)	247.2 (66.3)	0.26
Leg Press 3RM (kg)	150.6 (37.1)	165.0 (47.9)	68.8 (32.8)	57.4 (22.8)	0.85
Leg press volume (70% 1RM) (kg)	1636.2 (781.6)	1468 3 (578.0)	759.4 (558.1)	582.0 (298.4)	0.37

Note: Mean (*SD*). [1]P-values reported are for independent t-tests between intervention groups. BMI = body mass index, LBM = lean body mass, 1RM = 1 repetition maximum, 3RM = 3 repetition maximum.

Table 3. The effects of 12 weeks of IFT and CERT on body weight, BMI, body composition, pQCT and ultrasound variables in male and female participants. (Original to this manuscript)

	Group	Baseline	Week 12	Δ	Δ (%)	*P* (group)	*P* (time)	*P* (I)	*P* (S)
Body composition variables									
BMI (kg/m^2)	IFT Males (n=9)	26.6 (3.0)	25.1 (2.7)	-1.5	-5.6	0.98	<0.001[1]	0.64	0.007[3]
	CERT Males (n=8)	27.6 (2.4)	25.5 (2.7)	-2.1	-7.6				
	IFT Females (n=8)	27 5 (2.5)	26.5 (2.5)	-1.0	-3.6				
	CERT Females (n=9)	26.7 (3.36)	26.0 (2.9)	-0.7	-2.6				
Weight (kg)	IFT Males (n=9)	87.3 (12.5)	82.6 (11.7)	-4.7	-5.4	0.97	<0.001[1]	0.55	0.001[3]
	CERT Males (n=8)	88.0 (11.5)	81.7 (12.1)	-6.3	-7.2				
	IFT Females (n=8)	71.9 (10.7)	69.5 (9.8)	-2.4	-3.3				
	CERT Females (n=9)	72.1 (10.6)	70.1 (8.8)	-2.0	-2.8				
LBM (kg)	IFT Males (n=9)	64.4 (7.2)	65.7 (7.4)	1.3	2.0	0.99	<0.001[1]	0.47	0.003[3]
	CERT Males (n=8)	64.6 (9.4)	65.0 (9.6)	0.4	0.6				
	IFT Females (n=8)	43.0 (5.6)	45.4 (5.6)	2.4	5.6				
	CERT Females (n=9)	43 2 (4.8)	45.8 (4.1)	2.6	6.0				
Fat mass (kg)	IFT Males (n=9)	27.4 (8.9)	20.2 (6.3)	-7.2	-26.3	0.96	<0.001[1]	0.32	0.18
	CERT Males (n=8)	28 2 (5.0)	19.3 (4.8)	-8.9	-31.6				
	IFT Females (n=8)	32.8 (6.9)	26.0 (5.1)	-6.8	-20.7				
	CERT Females (n=9)	32 3 (8.8)	26.2 (7.9)	-6.1	-18.9				
Body fat percentage (%)	IFT Males (n=9)	29.4 (6.0)	23.1 (4.6)	-6.3	-21.4	0.95	<0.001[1]	0.64	0.75
	CERT Males (n=8)	30.4 (3.7)	22.8 (4.3)	-7.6	-25.0				
	IFT Females (n=8)	43.0 (3.9)	36.2 (2.2)	-6.8	-15.8				
	CERT Females (n=9)	42.2 (35.9)	35.9 (6.7)	-6.3	-14.9				
pQCT variables									
Muscle (cm^2)	IFT Males (n=7)	172 5 (23.2)	170.8 (23.4)	-1.7	-1.0	0.40	0.10	0.03[2]	0.21
	CERT Males (n=8)	162 5 (17.7)	165.2 (18.2)	2.7	1.7				
	IFT Females (n=8)	117 9 (21.3)	118.1 (17.8)	0.2	0.2				
	CERT Females (n=8)	111 3 (12.2)	118.1 (8.1)	6.8	6.1				
Intra-muscular fat (cm^2)[4]	IFT Males (n=7)	1.03 (0.64)	0.68 (0.58)	-0.35	-33.98	0.61	0.01[1]	0.63	0.70
	CERT Males (n=8)	2 30 (2.11)	1.33 (1.21)	-0.97	-42.17				
	IFT Females (n=8)	2 22 (1.08)	1.48 (0.78)	-0.74	-33.33				
	CERT Females (n=8)	1.71 (1.00)	1.40 (0.71)	-0.31	-18.13				
Subcutaneous fat (cm^2)	IFT Males (n=7)	71.2 (10.5)	63.7 (18.3)	-7.5	-10.5	0.41	<0.001[1]	0.62	0.83
	CERT Males (n=8)	79.7 (27.5)	64.9 (16.3)	-14.8	-18.6				
	IFT Females (n=8)	134.8 (35.8)	116.3 (33.8)	-18.5	-13.7				
	CERT Females (n=8)	142.6 (42.9)	136.6 (48.3)	-6.0	-4.2				
Ultrasound variables									
RF Thickness (cm)	IFT Males (n=7)	1.83 (0.36)	1.98 (0.31)	0 15	8.20	0.73	<0.001[1]	0.05	0.09
	CERT Males (n=5)	1 95 (0.27)	2.17 (0.28)	0 22	11.28				
	IFT Females (n=6)	1.78 (0.15)	1.82 (0.24)	0.04	2.25				
	CERT Females (n=9)	1.49 (0.26)	1.64 (0.27)	0 15	10.07				
RF CSA (cm^2)	IFT Males (n=7)	6 37 (1.09)	7.43 (1.37)	1.06	16.64	0.35	<0.001[1]	0.07	0.11
	CERT Males (n=5)	6.85 (1.50)	7.96 (1.32)	1 11	16.20				

	IFT Females (n=6)	6 16 (1.19)	6.19 (1.29)	0.03	0.49				
	CERT Females (n=9)	4 28 (1.23)	5.14 (1.46)	0.86	20.09				
RF EI (arbitrary units)	IFT Males (n=7)	22 1 (8.2)	15.9 (4.5)	-6.2	-28.1	0.43	<0.001[1]	0.47	0.95
	CERT Males (n=5)	17 1 (6.6)	14.5 (3.7)	-2.6	-15.2				
	IFT Females (n=6)	33.4 (14.0)	29.1 (15.0)	-4.3	-12.9				
	CERT Females (n=9)	42.0 (10.6)	38.3 (11.3)	-3.7	-8.8				
VI Thickness (cm)	IFT Males (n=7)	1 94 (0.37)	1.78 (0.20)	-0.16	-8.25	0.50	0.38	0.21	0.12
	CERT Males (n=5)	1.63 (0.22)	1.55 (0.36)	-0.08	-4.91				
	IFT Females (n=6)	1.47 (0.41)	1.42 (0.37)	-0.05	-3.40				
	CERT Females (n=9)	1.49 (0.37)	1.62 (0.22)	0 13	8.72				
VI EI (arbitrary units)	IFT Males (n=7)	38.4 (21.0)	35.6 (18.5)	-2.8	-7.3	0.99	0.75	0.65	0.90
	CERT Males (n=5)	33.5 (16.1)	35.1 (7.2)	1.6	4.8				
	IFT Females (n=6)	40.4 (10.5)	44.2 (29.2)	3.8	9.4				
	CERT Females (n=9)	48.4 (10.9)	41.8 (9.1)	-6.6	-13.6				

Note: Mean (*SD*). [1] Significantly different than baseline at week 12 in all groups combined. [2]Significant time x group interaction. [3]Significant time x sex interaction. [4]Data reported are untransformed; statistical analyses for intra-muscular fat are based on Log10 transformed values. BMI = Body Mass Index, CSA = cross-sectional area, EI = echogenicity, Group = main effect for diet group, I = time x group interaction, LBM = Lean Body Mass, RF = rectus femoris, S = time x sex interaction, Time = main effect for time, VI = vastus intermedius. pQCT was conducted on n = 31. Ultrasound was conducted on n = 27

Table 4. The effects of 12 weeks of IFT and CERT on energy and macronutrient intake in male and female participants. (Original to this manuscript)

Diet variable	Group	Baseline	During Intervention[3]	Δ	Δ (%)	*P* (group)	*P* (time)	*P* (I)	*P* (S)
Average daily energy intake (kJ)	IFT Males (n=9)	8134 (1993)	7678 (909)	-456	-5.6	0.32	0.006[1]	0.69	0.09
	CERT Males (n=8)	8222 (1930)	7981 (836)	-241	-2.9				
	IFT Females (n=8)	7041 (957)	5538 (646)	-1503	-21.3				
	CERT Females (n=9)	7469 (1939)	6219 (680)	-1250	-16.7				
Energy (kJ/kg)	IFT Males (n=9)	94 (25)	91 (12)	-3	-3.2	0.43	0.008[1]	0.69	0.03[2]
	CERT Males (n=8)	95 (27)	94 (10)	-1	-1.1				
	IFT Females (n=8)	100 (21)	79 (9)	-21	-21.0				
	CERT Females (n=9)	106 (32)	88 (13)	-18	-17.0				
Protein (g/kg)	IFT Males (n=9)	1 28 (0.52)	1.50 (0.22)	0.22	17.19	0.80	<0.001[1]	0.48	0.46
	CERT Males (n=8)	1.08 (0.33)	1.53 (0.27)	0.45	41.67				
	IFT Females (n=8)	1.05 (0.15)	1.31 (0.13)	0.26	24.76				
	CERT Females (n=9)	1 18 (0.38)	1.42 (0.15)	0.24	20.34				
Carbohydrate (g/kg)	IFT Males (n=9)	2 21 (0.86)	2.07 (0.59)	-0.14	-6.33	0.31	0.001[1]	0 51	0.01[2]
	CERT Males (n=8)	2 36 (0.71)	2.28 (0.42)	-0.08	-3.39				
	IFT Females (n=8)	2.46 (0.92)	1.61 (0.39)	-0.85	-34.55				
	CERT Females (n=9)	2 56 (0.66)	1.94 (0.36)	-0.62	-24.22				
Fat (g/kg)	IFT Males (n=9)	0.87 (0.34)	0.76 (0.16)	-0.11	-12.64	0.61	<0.001[1]	0 56	0.34
	CERT Males (n=8)	0 91 (0.33)	0.74 (0.16)	-0.17	-18.68				
	IFT Females (n=8)	1.05 (0.24)	0.73 (0.07)	-0.32	-30.48				
	CERT Females (n=9)	0 99 (0.33)	0.79 (0.17)	-0.20	-20.20				

Note: Mean (*SD*). [1] Significantly different than baseline at week 12 in all groups combined. [2]Significant time x sex interaction. [3]Average of week 1, 6 and 12 intakes. Group = main effect for diet group, I = time x group interaction, S = time x sex interaction, Time = main effect for time.

Table 5. Dietary intake for fasting and non-fasting days for IFT male and female participants. (Original to this manuscript)

Diet variable	Time	IFT Males Non-Fast Days (n = 9)	IFT Males Fast Days (n = 9)	IFT Females Non-Fast Days (n = 8)	IFT Females Fast Days (n = 8)
Average daily Energy Intake (kJ)	Week 1	9596 (1028)	2456 (136)	7185 (1041)	1957 (344)
	Week 6	9682 (1426)	2502 (431)	6758 (856)	1951 (260)
	Week 12	9380 (1712)	2511 (0)	6530 (1075)	1686 (292)[1]
	Overall average[2]	9619 (1058)	2490 (175)	6824 (734)	1865 (276)
Energy (kJ/kg)	Week 1	113 (14)	29 (4)	100 (19)	27 (4)
	Week 6	116 (22)	30 (5)	97 (16)	28 (4)
	Week 12	114 (24)	31 (4)	94 (14)	24 (5)
	Overall average[2]	114 (17)	29.62 (4.03)	97 (13)	26 (4)
Protein (g/kg)	Week 1	1.62 (0.40)	1.07 (0.15)	1.54 (0.19)	0.98 (0.12)
	Week 6	1.70 (0.39)	1.08 (0.21)	1.45 (0.28)	0.99 (0.16)
	Week 12	1.68 (0.41)	1.14 (0.16)	1.30 (0.21)	0.94 (0.17)
	Overall average[2]	1.63 (0.35)	1.11 (0.15)	1.44 (0.16)	0.98 (0.13)

Note: Mean (*SD*). [1] Significantly different to week 1 values in specified group, $p<0.05$. [2]Average of week 1, 6 and 12 intakes.

Table 6. The effects of 12 weeks of IFT and CERT on strength variables in male and female participants. (Original to this manuscript)

Strength variable	Group	Baseline	Week 12	Δ	Δ (%)	*P* (group)	*P* (time)	*P* (I)	*P* (S)
Bench press 3RM (kg)	IFT Males (n=9)	58.0 (12.1)	61.7 (11.3)	3.7	6.4	0.67	<0.001[1]	0 35	0.07
	CERT Males (n=8)	57.0 (12.9)	61.1 (13.9)	4 1	7.2				
	IFT Females (n=8)	26.8 (4.9)	31.9 (3.0)	5 1	19.0				
	CERT Females (n=9)	23.9 (30.8)	30.8 (5.2)	6 9	28.9				
Bench press volume (70% 1RM) (kg)	IFT Males (n=9)	611 (197)	692 (190)	81	13.3	0.29	<0.001[1]	0 29	0.46
	CERT Males (n=8)	522 (128)	657 (103)	135	25.9				
	IFT Females (n=8)	283 (104)	406 (61)	123	43.5				
	CERT Females (n=9)	247 (66)	394 (64)	147	59.5				
Leg Press 3RM (kg)	IFT Males (n=9)	150.6 (37.1)	185.0 (44)	34.4	22.8	0.95	<0.001[1]	0 51	0.34
	CERT Males (n=8)	165.0 (47.9)	186.8 (48)	21.8	13.2				
	IFT Females (n=8)	68.8 (32.8)	101.8 (33)	33.0	48.0				
	CERT Females (n=9)	57.4 (22.8)	93.9 (22)	36.5	63.6				
Leg press volume (70% 1RM) (kg)	IFT Males (n=9)	1636 (782)	2356 (1154)	720	44.0	0.36	<0.001[1]	0.74	0.95
	CERT Males (n=8)	1468 (578)	1904 (345)	436	29.7				
	IFT Females (n=8)	759 (558)	1269 (840)	510	67.2				
	CERT Females (n=9)	582 (1248)	1248 (526)	666	114.4				

Note: Mean (*SD*). [1] Significantly different than baseline at week 12 in all groups combined. Group = main effect for diet group, I = time x group interaction, S = time x sex interaction, Time = main effect for time, 1RM = 1 repetition maximum, 3RM = 3 repetition maximum.

Figures (Included as separate image files)

Figure 1. Study Flow Chart

Figure 2. Bivariate correlations between changes in body weight and LBM in CERT groups (**a**) and IFT groups (**b**); and between changes in percentage of total fat mass lost and LBM in CERT (**c**) and IFT (**d**) groups